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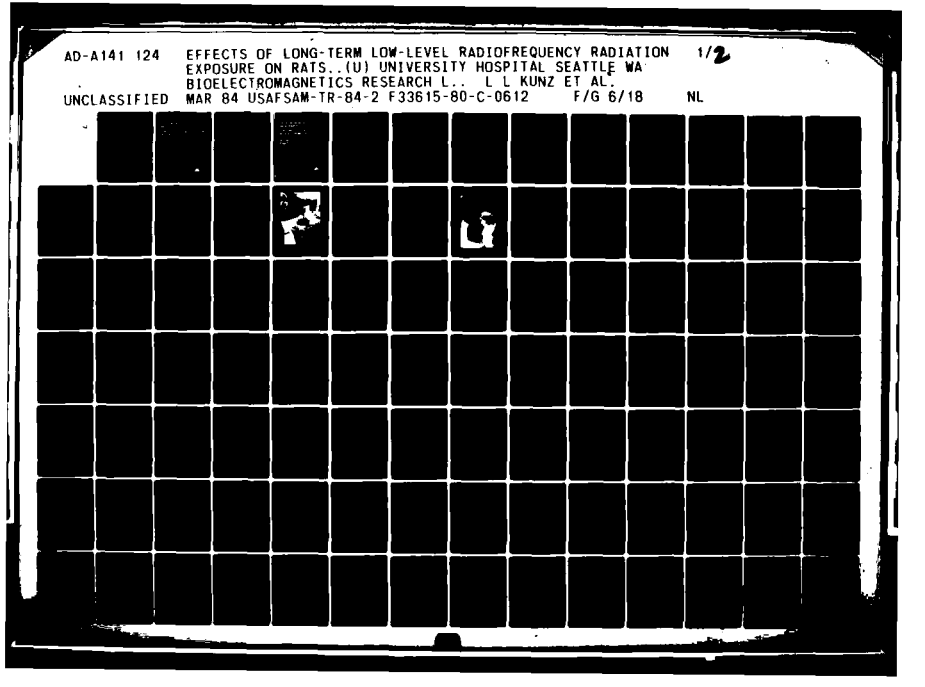

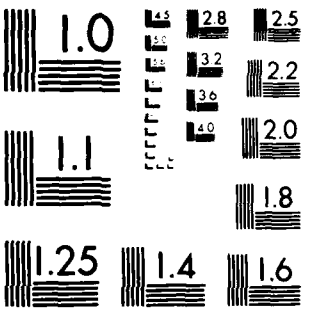

Report USAFSAM-TR-84-2

(12)

EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 6. HEMATOLOGICAL, SERUM CHEMISTRY, THYROXINE, AND PROTEIN ELECTROPHORESIS EVALUATIONS

Lawrence L. Kunz, D.V.M.
Robert B. Johnson, B.S.
Desmond Thompson, M.S.
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Bioelectromagnetics Research Laboratory
Department of Rehabilitation Medicine
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Seattle, Washington 98195

March 1984

Final Report for Period June 1980 - March 1983

Prepared for

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

NOTICES

This final report was submitted by the Bioelectromagnetics Research Laboratory, Department of Rehabilitation Medicine, School of Medicine, University of Washington, Seattle, Washington 98195, under contract F33615-80-C-0612, job order 7757-01-71, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks AFB, Texas. Dr. Jerome H. Krupp (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

Jerome H. Krupp

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The blood parameters were evaluated for rats exposed to 2450-MHz 480-~~μW/cm²~~ (10-~~μs~~ pulse, 800 pps, 8-Hz modulation) in circularly polarized waveguides for 21 hours a day for 25 months. The results of the hematology, serum chemistry, protein electrophoresis, and thyroxine evaluations show no significant points in time or on expected age-related baseline value changes.

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 6. HEMATOLOGICAL, SERUM CHEMISTRY, THYROXINE, AND PROTEIN ELECTROPHORESIS EVALUATIONS

INTRODUCTION

The increased use of microwave-emitting electronic devices for various purposes in consumer, military, medical, and industrial areas has resulted in the long-term low-level exposure of a significant proportion of the human population. Expertise in designing and manufacturing such devices has far exceeded biological research into the potential biohazardous risks from exposure to them. More than 6,000 articles about the biological effects of microwave radiation have been published; however, whether this exposure represents a human health hazard remains unclear (Czerski et al., 1974; Glaser and Dodge, 1975; Tyler, 1975; Justesen and Guy, 1977; Justesen and Baird, 1979; Gandhi, 1980). In most of the research projects, exposure durations have been relatively short and few animals have been exposed; thus, little insight has been gained into questions about potential long-term cumulative biological effects.

During the past three years, the Bioelectromagnetics Research Laboratory at the University of Washington has conducted the largest single evaluative study of the bioeffects of microwaves ever undertaken. The goal of the project was to investigate purported adverse health effects after long-term exposure to pulsed-microwave radiation. The major emphasis was to expose a large population of experimental animals to microwave radiation throughout their lifetime and, at the same time, to monitor them for cumulative effects on general health and longevity.

As part of this project, a unique exposure facility was constructed that allowed 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed in individual circularly polarized waveguides. This facility has been described in detail in Volume 1 of this report; we will only briefly discuss it here. The exposure facility consisted of two rooms, each containing 50 active-exposure waveguides plus 50 sham-exposure waveguides for control subjects. Each room contained two 2450-MHz pulsed-microwave generators, each capable of delivering a maximum of 10 W average power at 800 pps with a 10-μsec pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of 480 $\mu W/cm^2$. Whole-body calorimetry, thermographic analysis, and power-meter data analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

Two hundred 3-week-old male Sprague-Dawley rats obtained from a commercial barrier-reared colony were randomly assigned to exposed and sham-exposed treatment conditions. Exposure began at 8 weeks of age and continued for 25 months. Throughout this period, all surviving animals were sampled for blood at regular intervals; and serum chemistries, hematological values, protein electrophoretic patterns, thyroxin (T_4), and plasma corticosterone levels were determined. On a subpopulation of the exposed and sham-exposed populations, body weight and food and water consumption were measured daily and oxygen consumption and carbon dioxide production were measured periodically. Activity was assessed at regular intervals throughout the study in an open-field apparatus. After 13 months 10 rats from each treatment condition were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. At the end of 25 months, the 10 rats surviving from each group were killed and similar analyses were made. (For a detailed description of the scope of the project, see Volume 1 of this report.)

Many research projects assessing the biological effects and health hazards of microwave radiation have been concerned with single or limited biological endpoints. During the last few years, a multiple-panel profile has proven more useful to researchers for diagnosing and understanding abnormalities in their experimental animals. The use of automated

multiphasic analyzers, such as the Technicon SMAC computer-controlled biochemical analyzer, enables as many as 21 serum biochemical determinations on a small quantity of serum (600 µl) with reproducible precision and quality control. Radioimmunoassay techniques enable determination of T_4 levels from as little as 20 µl of blood, and the Beckman microzone electrophoresis chamber facilities provide protein electrophoresis from as little as 5 µl of blood. From only 0.3 ml of whole blood in EDTA, the Coulter counter can obtain the WBC and RBC, determine the PCV and hemoglobin, and perform a differential blood count.

The profile evaluation of experimental rats in our study has many advantages. The profile is an aid in evaluating the animals for unsuspected organ-system malfunction. In animals with subclinical or undiagnosed abnormalities, the profile can be of help in defining the problem. The emphasis is thus placed on correct interpretation of the profile results and the interrelation of different test results rather than on individual test results. The profile permits a better understanding of the pathophysiology of abnormal or disease states. As an organ system's abnormality or disease improves or worsens, changes in blood chemistry and hematology are monitored. The profile demonstrates multisystemic organ involvement, which is often missed if only individual tests are selected and conducted. The profile also indicates the full significance of a particular abnormality and provides a data base for presumptive and definitive diagnoses.

This report is the sixth of a series (major subtopics of interest) concerning the chronic-exposure study. It covers the conduct and results of evaluations of hematology, serum chemistry, protein electrophoretic patterns, and T_4 content of the exposed and sham-exposed animals.

BLOOD SAMPLING

The research protocol required the rapid collection of blood from all test animals in a 2-h period per day over 4 days for each blood sampling. The collection procedure was designed to be as atraumatic and rapid as possible. To prevent artifactual elevation of corticosterone, blood samples for serum corticosterone must be drawn within 2 min after a rat is removed from its caging situation (Zimmerman and Critchlow, 1967; Davidson et al., 1968). In a specifically designed chamber the animals were rapidly anesthetized with a mixture of halothane, nitrous oxide, and oxygen; and blood samples were drawn by using the relatively atraumatic retro-orbital technique. Alternate eyes were sampled for blood in successive sampling sessions so as to minimize ocular damage. A single blood sample, 1.8 to 2.0 ml, was taken to serve for all determinations.

The first sampling was done 4 weeks prior to initiation of microwave exposure, and data from this sampling were interpreted as baseline measures. The second sampling was during the seventh week of exposure. During the first year of the study, the rats were sampled for blood every 6 weeks for 9 samplings, and then every 12 weeks.

This frequency of bleeding is sufficient for detecting the onset of most degenerative or disease states that may occur during the life of a rat but yet, in our experience, does not unduly stress the animal. This frequency of biochemical evaluation also increases the opportunity to detect subclinical abnormalities and follow their pathophysiological course.

Hematology and serum chemistry were evaluated in blood collected during the second sampling; and when serum was adequate, the corticosterone level also was determined. Subsequently, hematology, serum chemistry, and protein electrophoretic patterns were evaluated on each sample every 6 weeks, and corticosterone and T_4 levels were determined every 12 weeks (every other sixth-week sampling). After the tenth sampling, corticosterone samples were no longer taken, and the interbleeding interval was lengthened to 12 weeks. Corticosterone was assayed also at the last sampling prior to and again at the time of the terminal kill.

Anesthesia

Contrary to reported findings that blood collection from unanesthetized rats is feasible (Miqdalof, 1976; Noller, 1955; Sorq and Buckner, 1964; and Stone, 1954), we have found that an unanesthetized adult male rat is quite strong and virtually impossible to adequately restrain by hand if the procedure is to be successfully used on a chronic basis. The struggling of the animal greatly increases the chances of trauma to the eye and surrounding structures, not to mention the inherent stress imposed by such restraint.

Consequently, a system for lightly anesthetizing the animal was devised. A design similar to that described by Luschei and Mehaffey (1967), with a vaporizing apparatus, was constructed (Fig. 1). Pressurized oxygen was "bubbled" through liquid halothane (Fluorothane, Ayerst Laboratories Inc., New York, NY 10017) in a gas-washing bottle (Pyrex, 250 ml). The vapor was then combined with oxygen and nitrous oxide, and the mixture was delivered to a variable-volume chamber in which the rat was contained (Fig. 2). The main oxygen and nitrous oxide flows and the partial flow (by shunt) of oxygen through the halothane were monitored with separate flowmeters (Manostat, #36-541-03) and regulated to yield a 2-3% concentration of halothane and a 33% concentration of nitrous oxide in oxygen delivered to the chamber. The volume of the chamber could be altered by adjusting the height of the plunger to allow for rat growth during the lifetime study. The vaporizer system was an open-flow system with exhaust connected to the exhaust ventilation in the room. The animal was adequately anesthetized within 1 min, and the blood was withdrawn during the next 30 s--a timespan well within the accepted limit for samples used in corticosterone analyses. Recovery from anesthesia occurred almost immediately after completion of the sampling. The nonflammable and nonexplosive qualities of halothane, in addition to its high safety margin and relatively innocuous effects at this low dose, made it the anesthetic agent of choice for our purposes (Burnap et al., 1958; Cook and Dorman, 1969; Morch and Jobgen, 1959; Parbrook, 1967; Sebesteny, 1971; Stephen et al., 1958; Van Dyke and Chenoweth, 1965; and Virtue et al., 1958).

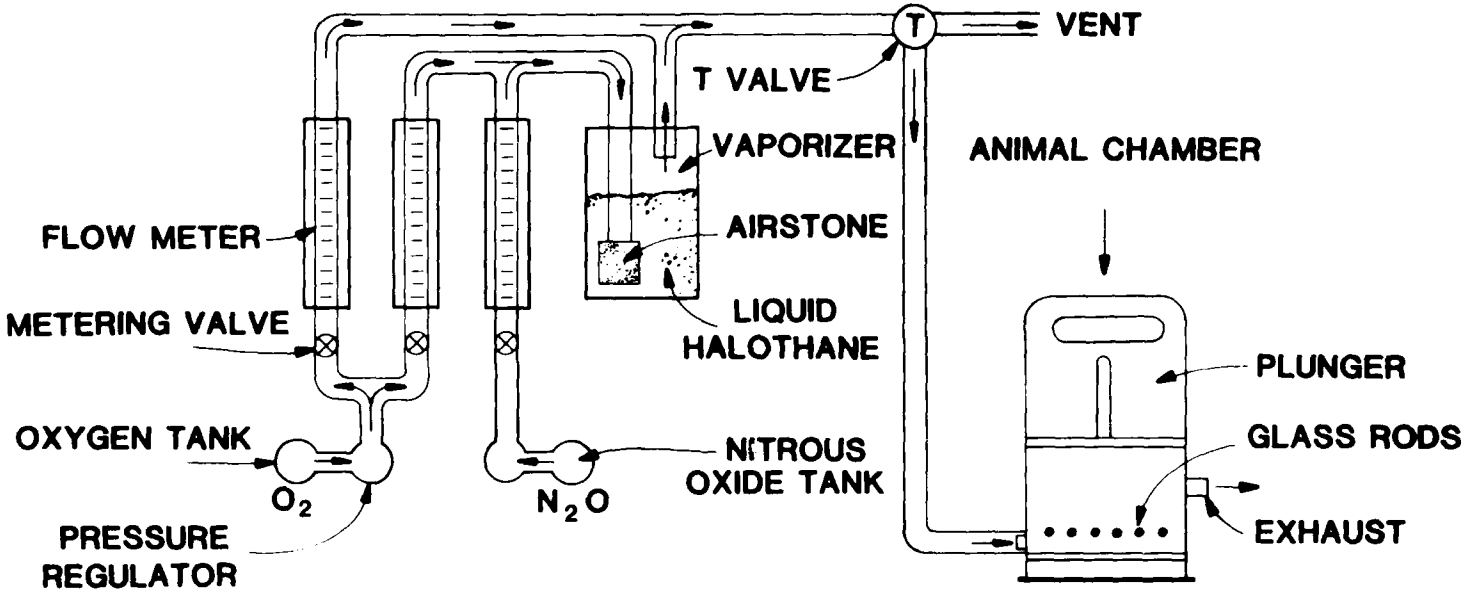

Fig. 1. Schematic of the adjustable-volume halothane-anesthesia chamber.

Blood-Serum Collection

The retro-orbital technique for collecting blood samples from laboratory rats was chosen for this project. One advantage this method has over others is that it apparently has no adverse effects on the animal, and the long-term survival of the animals was of utmost importance. Another advantage is the quality of the sample obtained. The volume and quality of samples obtained from the orbital venous plexus remained consistent among animals and over repeated samplings during the project.

Five technicians were required to successfully perform the rapid sampling of the large number of rats (50 in 2 h) in the context of the daily maintenance procedures. The sampler was directly assisted by the technician responsible for anesthesia, and another handled the blood samples. A fourth technician delivered the animals to the anesthesia technician and returned them to their cage after the sampling. The fifth participant, a blood processor, was responsible for centrifuging the samples.

The typical sequence of events was as follows:

1. Prior to sampling and after "unhousing," the rats were allowed to remain in their holding bins for 30 min.

2. A rat was delivered to the anesthesia technician, who placed it in one of two identical anesthetic chambers.

3. The anesthesia technician carefully watched the rat for slow rhythmic abdominal breathing--an indication of sufficient anesthesia for the sampling procedure.

4. The anesthetized rat was removed from the chamber and handed to the blood sampler.

5. The anesthesia technician then placed the next rat in the other chamber. The chamber used for the previous rat was wiped with a disinfectant while the second rat was being anesthetized.

6. To bleed the right eye, in the left hand the technician held the rat such that its hind feet rested on the counter top. Sampling was performed on alternate eyes every session. (Bleeding of the left eye required a repositioning of the animal, but the basic procedure was retained). The left thumb was positioned under the rat's right foreleg and onto the jaw under the right eye, while the left index finger was held on top of the head above the eye. (See Fig. 3.) The skin surrounding the eye was then gently retracted by thumb and forefinger, causing the eye to bulge slightly. The tip of the pipette (our greatest success was with commercially available Pasteur pipettes) was then inserted into the anterior canthus of the eye, rotated, and forced through the nictitating membrane between the eyeball and the orbit at a relatively steep angle (35-40 degrees), roughly perpendicular to the plane defined by the side of the head through the eye. Once the pipette was through the membrane, the angle was decreased and the pipette advanced toward the back of the eye. After rupture of the venous plexus, blood was drawn into the pipette via capillary action. To maintain an even flow and facilitate rapid sampling, the technician further decreased the angle of the pipette and rotated the rat's head so that gravitational pull was utilized in filling the enlarged portion of the pipette. Advancing or withdrawing the pipette slightly was also sometimes helpful. During the sampling the left hand merely afforded minimal support for the rat, as applying unnecessary pressure (i.e., squeezing) might impede flow of blood into the pipette. When 2 ml of blood had been obtained, the pipette was removed and handed to the assistant. Simultaneously, the rat was lowered to the work surface and light pressure was applied to the closed eye for 5-10 s to stop the bleeding. The eye was then swabbed with 0.9% physiologic saline solution. The rat was inspected for obvious signs of adverse effects before being returned to its holding bin. The eye was again examined before rehousing later in the day.

7. Upon receipt of the full pipette, the assistant immediately dropped approximately 0.3 ml into a 3-ml B-D Vacutainer (Becton-Dickinson Co., Rutherford, NJ 07070) containing 4.5 mg EDTA. The Vacutainer was then turned over to the blood sampler, who rotated it gently to preclude clotting; this subsample was used for the hematology studies. The assistant then divided the remaining sample between two 600-µl, plastic B-D Serum Separator Microtainers. These tubes were capped and given to the processor for centrifugation.

8. The processor allowed the sample to clot for at least 30 min and then spun it for 7 min at high speed in an Eppendorf Micro Centrifuge, model 5412 (Brinkman Instruments, Westbury, NY).

The blood samples in the B-D serum separator tubes were used for determinations of serum chemistry, corticosterone, and T_4. These microtainer tubes contain an inert silicone material with a specific gravity between that of serum and blood cells. During centrifugation of the clotted specimen, the silicone gel rises to the serum-cell interface, where it forms a physical barrier separating the serum from the cell clot. This provides an excellent method of obtaining a maximum yield of high-quality serum. After centrifugation the serum was stored under refrigeration in individual glass vials until analysis.

The silicone-gel interface and the rapid transfer of serum into a separate vial was required because prolonged exposure of serum to red cells, especially at room temperature, will result in increases in lactate dehydrogenase (LDH) and serum-glutamic-oxaloacetic transaminase (SGOT) and a fall in glucose, calcium, and carbon dioxide as a result of continued cell metabolism or breakdown. The retro-orbital blood-collection technique used produces very little hemolysis, an important characteristic because samples with hemolysis show significant increases in LDH and SGOT and some increase in serum-glutamic-pyruvic transaminase (SGPT) and total protein. The following constituents have a minor decrease in value in hemolyzed samples: alkaline phosphatase, total bilirubin, glucose, and calcium.

HEMATOLOGY

Progress in delineating the hematology of the rat has continued over the past several years, but much of the data remains fragmentary and incomplete. Hematological values vary owing to stock or strain and source of rats (Ringler and Dahich, 1979); thus, it was important in this study that baseline values be established from sham-exposed controls to serve as standards for evaluating exposed animals. Hematological results improve in reliability with sample size (number of animals per experimental group) and control of environmental, sampling, and analytical variables. The accuracy and reproducibility of data also improve with the use of microtechniques in conjunction with the Coulter electronic counter.

Methods

The following hematological parameters, indices, and differential counts were determined from the 0.3- to 0.5-ml sample of EDTA and whole blood:

white blood cell count (WBC)
red blood cell count (RBC)
hematocrit (HCT)
hemoglobin (Hgb)
mean corpuscular volume (MCV)
mean corpuscular hemoglobin (MCH)
mean corpuscular hemoglobin concentration (MCHC)
neutrophils
lymphocytes
eosinophils
monocytes

A model ZBI Coulter (Coulter Electronic, Inc., Hialeah, FL) blood cell counter was used for the WBC and RBC counts. Heparinized microhematocrit tubes were used for determining the hematocrit. Hemoglobin was determined by the cyanmethemoglobin method, and trained hematology technicians performed the differential counts.

The blood film was prepared on a slide from a thoroughly mixed sample. The film was then stained by Wright's method and evaluated microscopically. The hematology technicians and their work were supervised and reviewed by a board-certified veterinary pathologist. The evaluation of blood cell morphology is subjective and rests on the judgment of the technologist. A semiquantitative reporting system was designed to serve as a standardized procedure for consistency in morphological determinations; guidance in its development was provided by Walton (1973), Napoli et al. (1980), and Lloyd (1982).

Results

Bar-graph comparisons of all hematological parameters and indices for all sampling sessions are presented in Figs. 4 through 10; in Figs. 11 through 14, the absolute cell counts for lymphocytes, neutrophils, eosinophils, and monocytes. Data for all parameters were statistically analyzed; summaries of the results (including the mean, maximum, and minimum and standard deviation and error for each parameter) are presented in Tables 1 through 11.

Although many of the distributions were positively skewed, in many instances the value was zero; we therefore chose to analyze the data in the original scale without transformation. Examination of the correlation matrices for these parameters revealed uniformly low run-to-run correlations, without systematic pattern. Within any one run, correlations between the 11 parameters were also uniformly low.

Since complete data were not available for all animals throughout all 15 sessions (owing to mortality), multivariate analyses with the Hotelling T^2-statistic were performed on a truncated data set from animals with complete data records for sessions 2 through 8. (Session 1 was not included since it was prior to the actual start of exposure conditions.) Over 70% of all animals had complete data for these sessions. None of these analyses indicated an overall difference between exposed and sham-exposed populations over all six sessions for any of the 11 parameters considered.

All parameters for each of the 15 sampling sessions were also compared by individual *t*-tests. These indicated that the exposed population had a significant reduction in absolute eosinophil count during session 2 (t = -2.96, p = .0033, df = 195) and marginally significant reductions in absolute neutrophil count during sessions 2 (t = -1.83, p = .0684, df = 195) and 3 (t = -1.96, p = .052, df = 194). None of the other individual comparisons were significant.

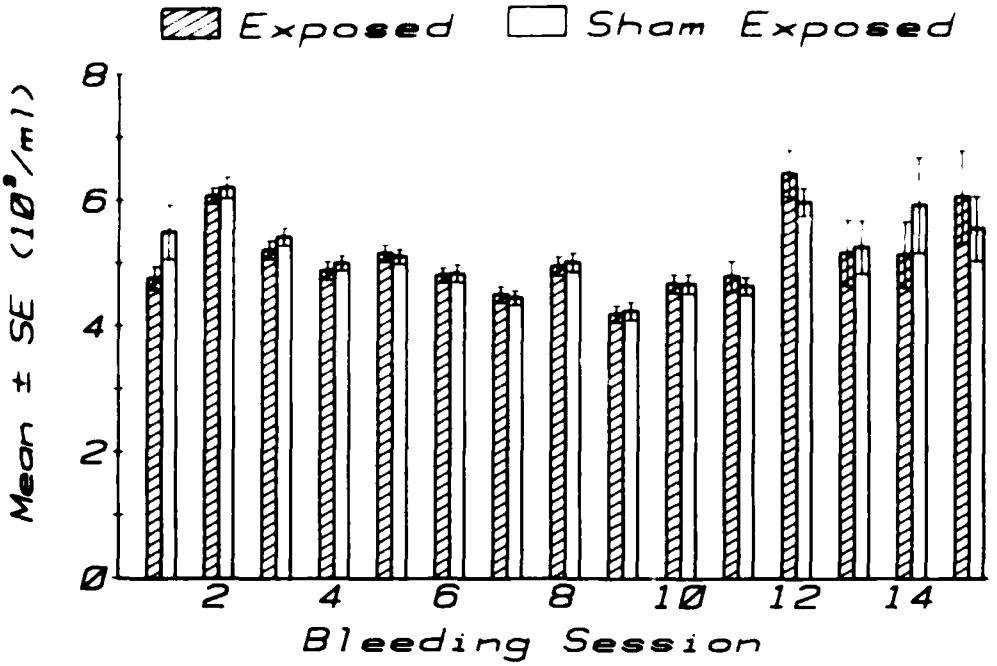

Fig. 4. Comparison of WHITE CELL COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

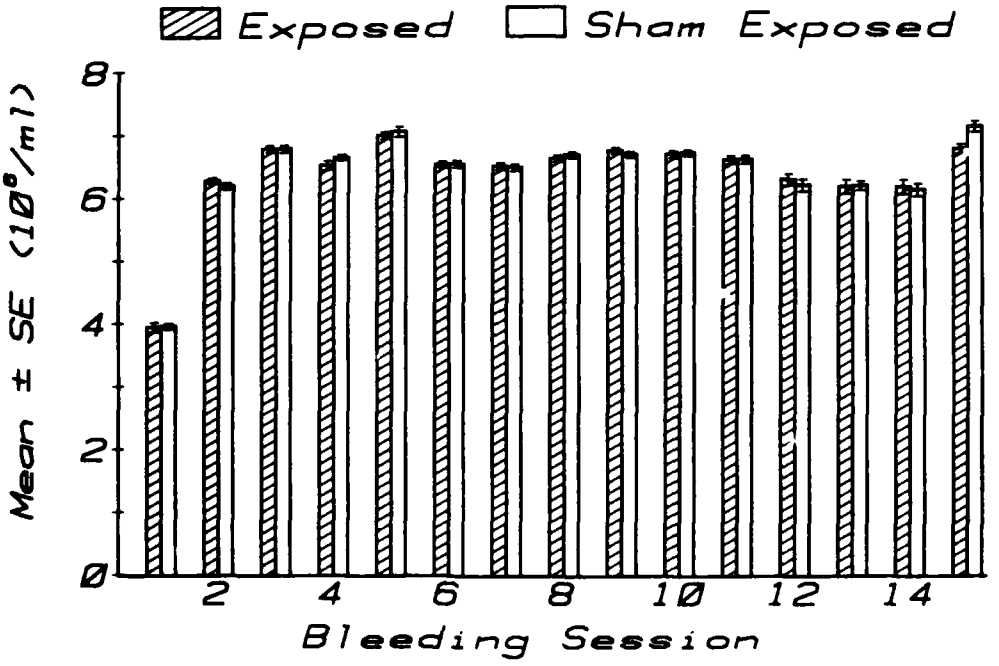

Fig. 5. Comparison of RED CELL COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

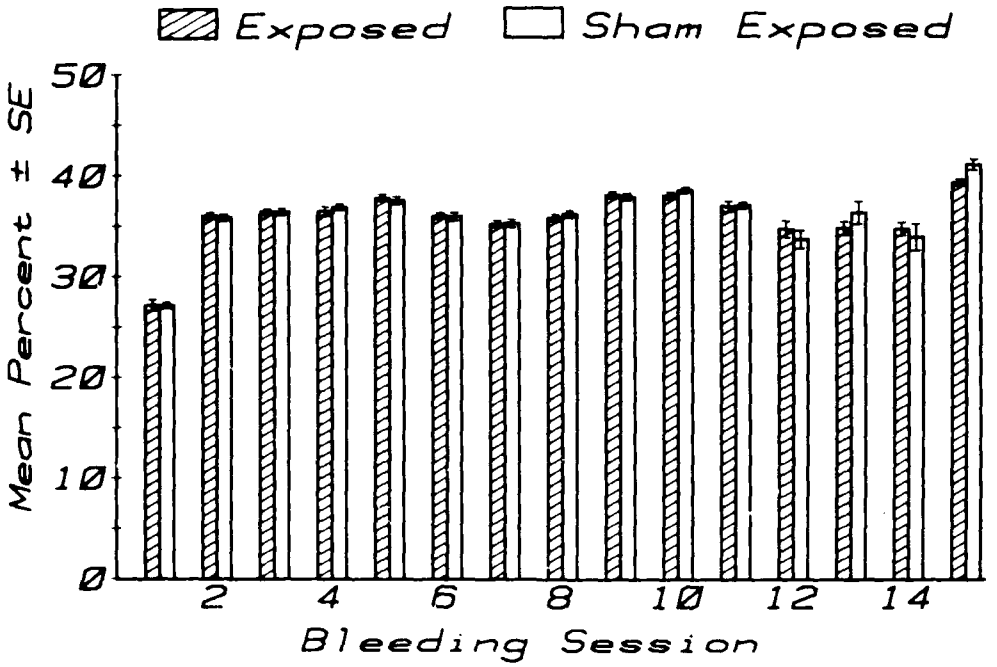

Fig. 6. Comparison of HEMATOCRIT from exposed and sham-exposed animals throughout 15 sampling sessions.

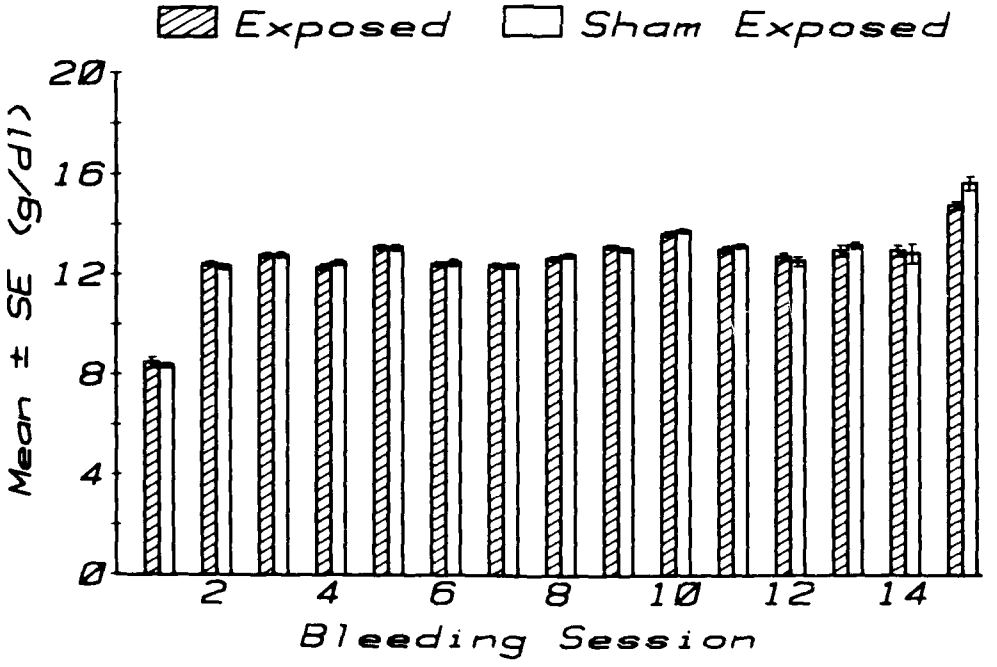

Fig. 7. Comparison of HEMOGLOBIN from exposed and sham-exposed animals for 15 sampling sessions.

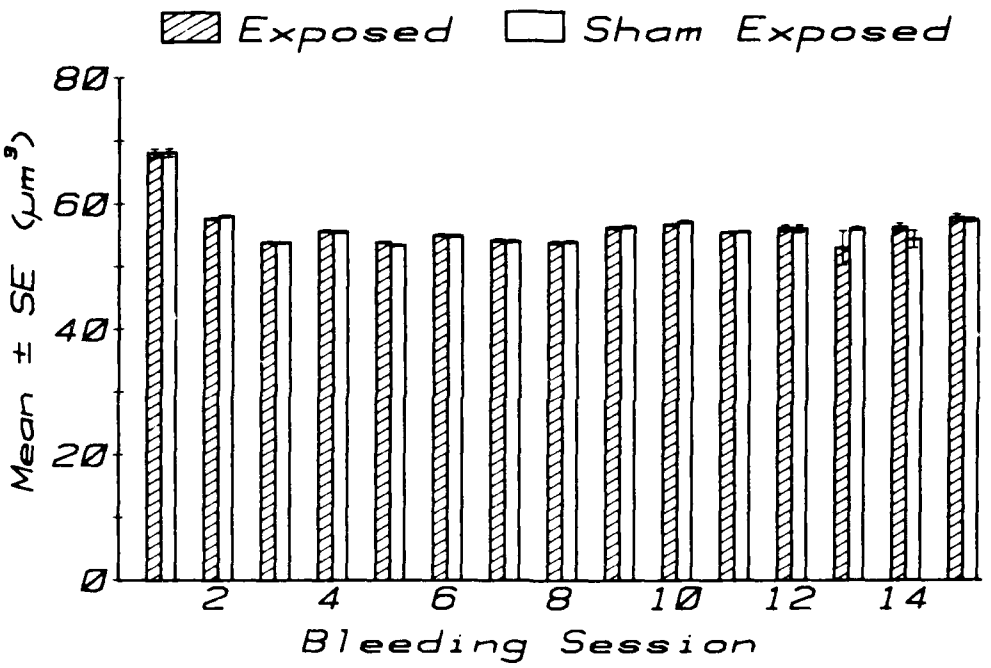

Fig. 8. Comparison of MEAN CELL VOLUMES for exposed and sham-exposed animals for 15 sampling sessions.

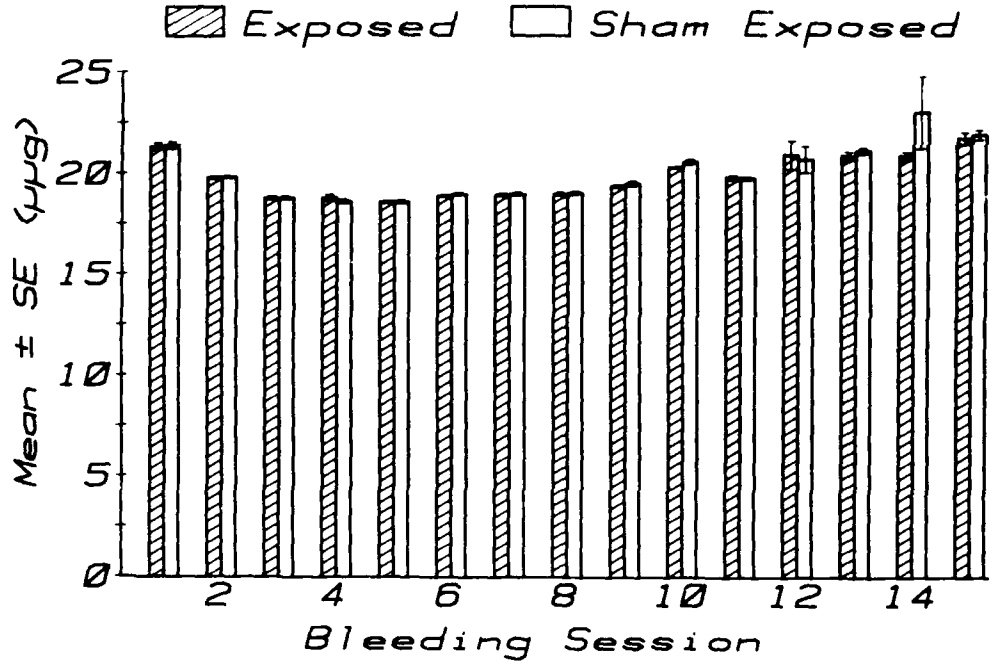

Fig. 9. Comparison of MEAN CORPUSCULAR HEMOGLOBIN in samples from exposed and sham-exposed animals for 15 sampling sessions.

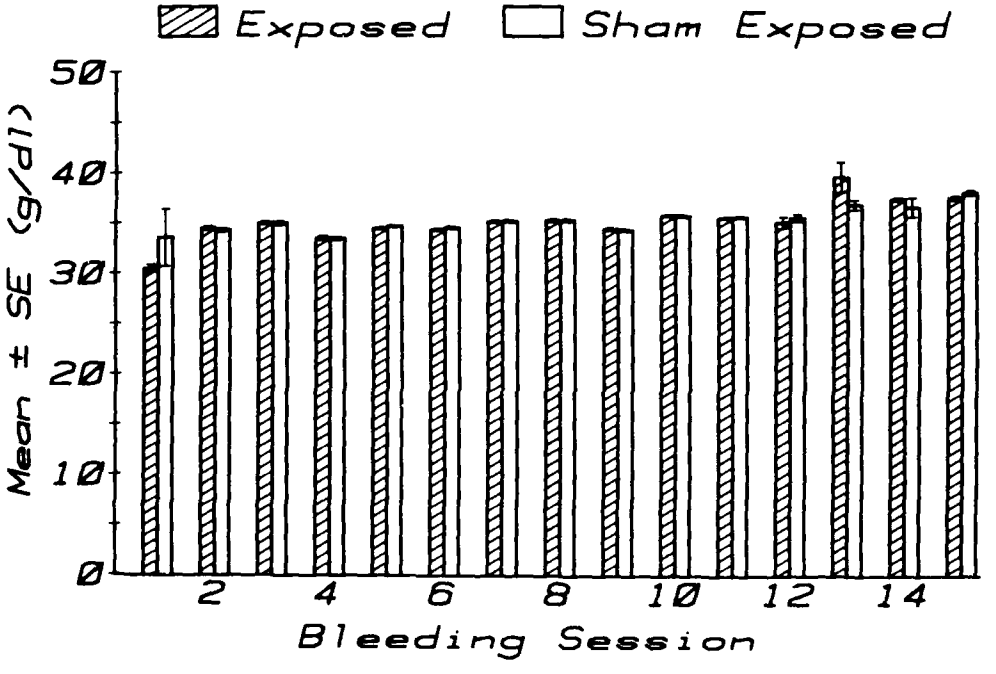

Fig. 10. Comparison of MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATIONS for exposed and sham-exposed animals for 15 sampling sessions.

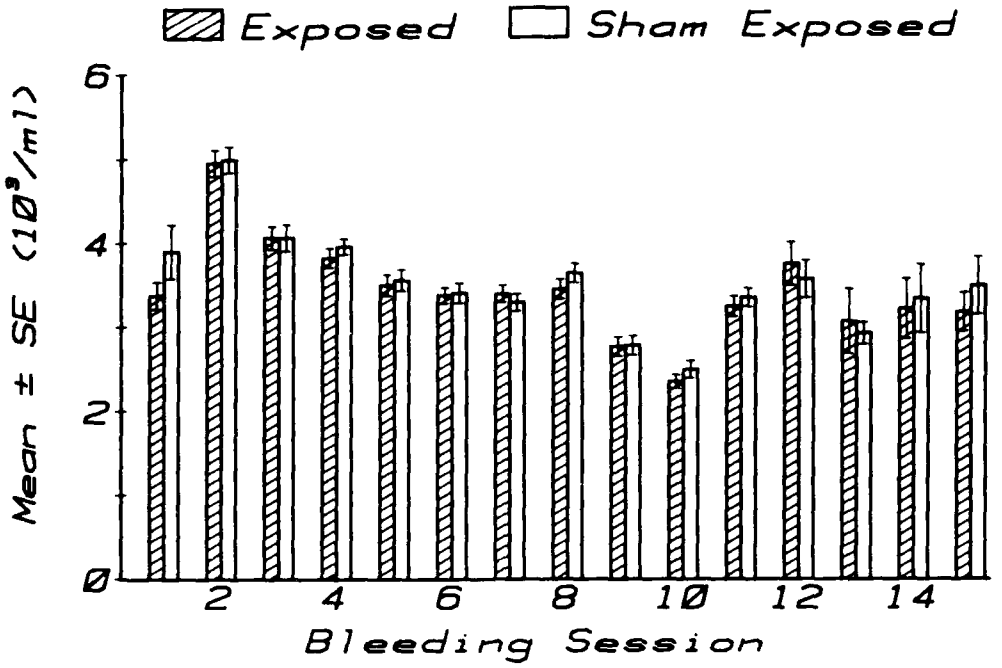

Fig. 11. Comparison of LYMPHOCYTE COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

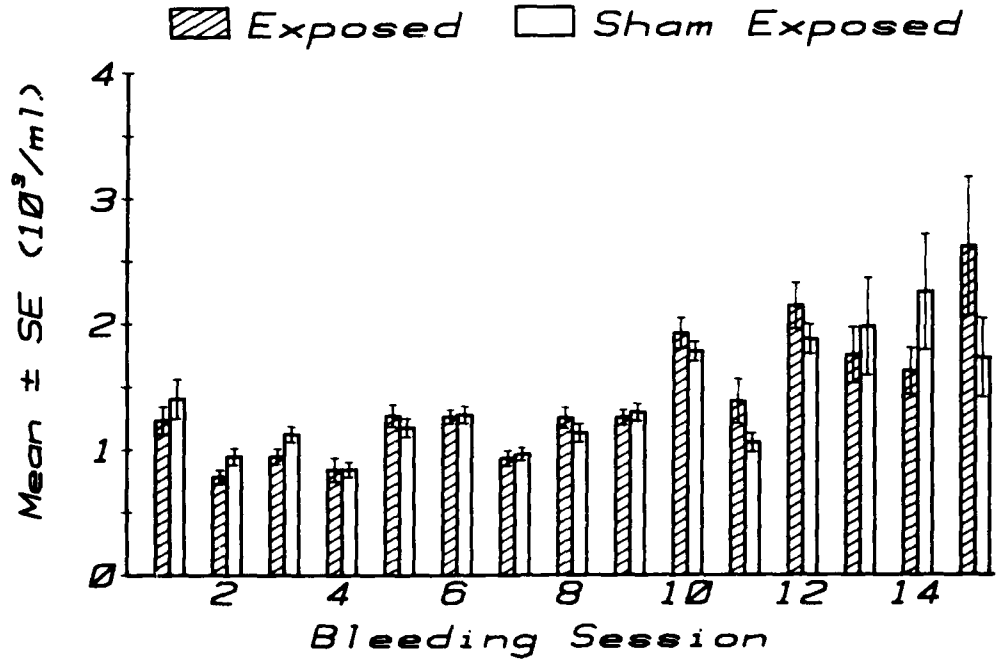

Fig. 12. Comparison of NEUTROPHIL COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

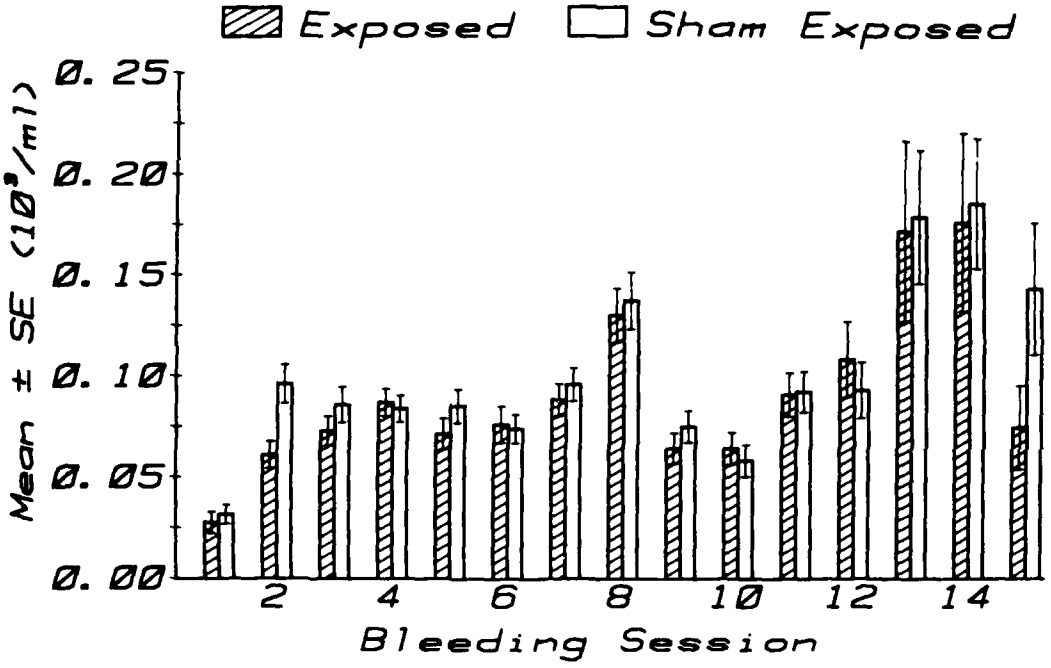

Fig. 13. Comparison of EOSINOPHIL COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

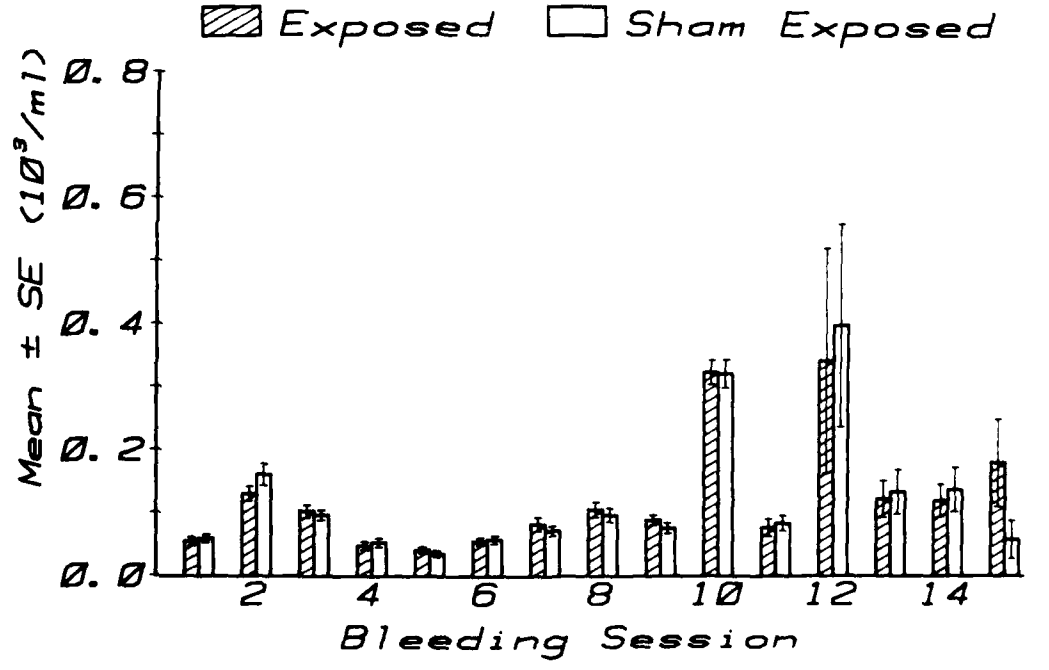

Fig. 14. Comparison of MONOCYTE COUNTS for exposed and sham-exposed animals for 15 sampling sessions.

TABLE 1. RESULTS OF STATISTICAL ANALYSIS OF WHITE CELL COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	4.77	4.30	3.90	1.82	1.7	10.5	0.19
2	100	6.08	6.20	6.20	1.38	2.9	9.1	0.14
3	98	5.21	5.20	5.90	1.50	1.5	8.9	0.15
4	95	4.88	4.50	4.00	1.49	2.0	10.6	0.15
5	94	5.16	5.15	5.40	1.36	1.8	8.8	0.14
6	93	4.81	4.80	5.50	1.21	2.1	7.7	0.12
7	92	4.50	4.50	4.30	1.30	0.0	8.8	0.13
8	94	4.94	5.00	5.00	1.54	2.0	11.8	0.16
9	90	4.18	4.10	4.10	1.34	1.8	9.1	0.14
10	90	4.66	4.45	4.70	1.48	2.2	12.1	0.16
11	72	4.79	4.45	6.30	2.11	2.0	14.8	0.25
12	51	6.41	5.90	6.20	2.77	0.0	15.2	0.38
13	18	5.15	4.80	3.10	2.31	2.8	12.4	0.53
14	18	5.13	4.80	3.10	2.32	2.8	12.4	0.53
15	12	6.04	5.05	5.40	2.68	3.4	11.6	0.74

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	5.50	4.60	4.70	4.40	1.8	42.1	0.44
2	97	6.21	6.20	6.80	1.77	3.1	10.9	0.18
3	98	5.42	5.40	5.40	1.48	2.2	9.2	0.15
4	95	5.00	4.70	4.30	1.22	3.0	8.0	0.12
5	90	5.10	5.00	5.00	1.20	2.7	8.7	0.13
6	85	4.83	4.70	5.20	1.35	1.9	8.4	0.15
7	90	4.44	4.40	5.20	1.17	2.6	7.2	0.12
8	88	5.00	4.80	5.10	1.48	2.6	9.2	0.16
9	86	4.22	4.20	3.90	1.41	1.3	9.5	0.15
10	81	4.67	4.50	4.50	1.44	2.7	14.0	0.16
11	66	4.63	4.40	4.40	1.19	2.2	7.9	0.15
12	54	5.95	6.15	7.00	1.73	2.3	12.8	0.23
13	18	5.24	4.80	4.80	1.87	3.4	11.9	0.43
14	18	5.91	4.80	4.80	3.34	3.4	17.0	0.77
15	11	5.54	5.90	4.00	1.80	2.3	8.2	0.52

TABLE 2. RESULTS OF STATISTICAL ANALYSIS OF RED CELL COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	3.96	3.98	4.40	0.80	1.3	9.2	0.08
2	100	6.30	6.33	6.33	0.57	4.5	8.1	0.06
3	98	6.81	6.89	7.25	0.63	5.1	8.2	0.06
4	95	6.55	6.61	6.62	0.78	3.8	8.7	0.08
5	94	7.02	7.13	7.49	0.66	4.8	8.2	0.07
6	93	6.56	6.56	6.77	0.58	4.7	7.6	0.06
7	92	6.52	6.60	6.80	0.63	3.5	7.9	0.07
8	94	6.65	6.75	6.73	0.60	5.0	8.0	0.06
9	90	6.78	6.79	7.22	0.54	4.9	7.9	0.06
10	90	6.71	6.81	7.41	0.60	4.9	7.8	0.06
11	72	6.63	6.77	7.20	0.63	3.7	7.8	0.07
12	51	6.32	6.49	5.90	0.73	3.3	7.4	0.10
13	18	6.20	6.20	5.60	0.52	5.2	7.2	0.12
14	18	6.20	6.20	6.50	0.54	5.2	7.2	0.12
15	12	6.80	6.80	6.80	0.29	6.2	7.2	0.08

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	3.98	4.10	4.10	0.53	1.4	5.1	0.05
2	97	6.22	6.38	6.48	0.63	3.7	7.3	0.06
3	98	6.81	6.93	7.57	0.68	4.8	7.9	0.07
4	95	6.67	6.69	6.87	0.54	5.3	7.7	0.05
5	90	7.08	7.14	7.42	0.83	3.9	10.6	0.09
6	85	6.56	6.64	7.02	0.62	4.8	8.7	0.07
7	90	6.50	6.53	7.06	0.60	4.3	7.5	0.06
8	88	6.71	6.72	7.01	0.53	5.5	7.9	0.06
9	86	6.72	6.72	6.89	0.52	4.2	7.8	0.06
10	81	6.74	6.76	7.47	0.50	5.0	7.7	0.06
11	66	6.64	6.64	6.55	0.57	5.0	7.8	0.07
12	54	6.23	6.29	6.75	0.78	1.9	7.3	0.11
13	18	6.22	6.27	6.60	0.36	5.4	6.8	0.08
14	18	6.15	6.27	6.60	0.47	4.9	6.8	0.11
15	11	7.15	7.20	7.20	0.33	6.6	7.7	0.09

TABLE 3. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM HEMATOCRIT FROM EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	27.20	27.60	28.40	5.40	10.2	63.0	0.57
2	100	36.07	36.45	39.00	3.51	25.9	48.4	0.35
3	98	36.44	36.60	34.60	3.26	27.8	43.0	0.33
4	95	36.47	36.90	40.00	4.54	21.3	47.0	0.46
5	94	37.76	38.35	40.00	3.68	26.0	44.5	0.38
6	93	35.97	36.10	38.00	3.29	26.4	42.4	0.34
7	92	35.12	35.10	37.40	3.54	22.1	44.0	0.37
8	94	35.75	35.75	34.60	3.50	26.2	43.7	0.36
9	90	38.04	37.90	39.70	3.30	28.8	46.8	0.35
10	90	37.95	38.20	41.00	3.58	27.3	46.1	0.37
11	72	36.90	37.30	37.30	4.54	18.5	54.9	0.53
12	51	34.63	35.80	36.90	5.98	0.0	41.5	0.83
13	18	34.69	34.60	34.40	2.90	28.7	41.2	0.66
14	18	34.62	34.60	35.50	2.86	28.7	41.2	0.65
15	12	39.22	38.95	41.20	1.28	37.6	41.2	0.35

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	27.19	27.90	29.00	3.13	14.3	34.9	0.32
2	97	35.88	36.60	37.90	3.58	22.9	43.0	0.36
3	98	36.44	36.60	37.40	3.66	28.4	43.6	0.37
4	95	36.88	37.00	36.70	3.20	28.1	42.5	0.33
5	90	37.53	38.00	41.00	3.87	23.0	45.0	0.41
6	85	35.92	36.40	39.00	3.84	26.3	49.8	0.41
7	90	35.21	35.55	40.00	3.91	23.3	52.5	0.41
8	88	36.14	36.20	38.90	3.44	28.7	44.5	0.36
9	86	37.83	37.75	37.50	3.46	25.4	48.2	0.37
10	81	38.47	38.60	39.50	2.82	31.4	44.4	0.31
11	66	36.97	37.25	39.40	2.76	30.6	42.2	0.34
12	54	33.63	35.00	38.60	6.72	0.0	40.4	0.91
13	18	36.24	35.45	35.20	4.90	30.0	53.7	1.12
14	18	33.86	35.20	35.20	5.76	12.3	38.4	1.32
15	11	40.99	41.10	40.30	1.96	37.3	44.1	0.56

TABLE 4. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM HEMOGLOBIN FROM EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	8.49	8.50	9.00	2.29	3.0	27.2	0.24
2	100	12.45	12.55	13.50	1.11	8.5	14.5	0.11
3	98	12.77	12.95	13.30	1.22	9.0	15.7	0.12
4	95	12.29	12.40	12.40	1.49	7.1	16.3	0.15
5	94	13.07	13.30	13.80	1.30	8.6	15.8	0.13
6	93	12.38	12.40	12.00	1.17	8.6	14.6	0.12
7	92	12.34	12.50	13.20	1.13	7.3	14.2	0.12
8	94	12.63	12.75	12.70	1.19	9.2	15.7	0.12
9	90	13.12	13.10	13.60	1.00	9.9	15.0	0.11
10	90	13.63	13.70	13.50	1.25	10.1	15.7	0.13
11	72	13.01	13.20	13.20	1.38	6.9	15.1	0.16
12	51	12.71	13.00	13.30	1.28	8.0	14.8	0.18
13	18	12.95	13.05	13.10	1.06	10.7	15.4	0.24
14	18	12.95	13.05	13.10	1.06	10.7	15.4	0.24
15	12	14.73	14.60	14.60	0.65	13.8	16.2	0.18

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	8.39	8.70	8.80	1.07	3.1	11.0	0.11
2	97	12.33	12.60	12.80	1.30	7.3	16.4	0.13
3	98	12.77	12.90	12.80	1.28	9.2	16.4	0.13
4	95	12.47	12.40	13.50	1.31	9.6	17.7	0.13
5	90	13.05	13.10	14.10	1.32	9.0	15.5	0.14
6	85	12.46	12.70	13.50	1.40	8.8	17.9	0.15
7	90	12.34	12.40	13.30	1.26	8.3	15.0	0.13
8	88	12.77	12.85	14.00	1.08	10.2	15.5	0.11
9	86	13.00	13.10	13.70	1.07	8.3	15.7	0.11
10	81	13.75	13.90	14.20	0.97	11.3	15.4	0.11
11	66	13.14	13.20	13.50	0.91	11.2	15.1	0.11
12	54	12.53	12.65	12.30	1.55	4.0	14.6	0.21
13	18	13.15	13.20	14.00	0.67	11.8	14.2	0.15
14	18	12.84	13.30	14.00	1.80	6.1	14.2	0.41
15	11	15.63	15.60	15.40	1.01	13.6	17.0	0.29

TABLE 5. RESULTS OF STATISTICAL ANALYSIS OF MEAN CELL VOLUMES FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	68.11	68.00	70.00	6.93	21.1	90.0	0.73
2	100	57.63	58.00	58.00	1.93	51.0	64.0	0.19
3	98	53.81	54.00	53.00	1.93	50.0	60.0	0.19
4	95	55.63	56.00	57.00	2.17	50.0	63.0	0.22
5	94	53.81	54.00	55.00	1.92	50.0	60.0	0.20
6	93	54.91	55.00	55.00	2.20	51.0	60.0	0.23
7	92	54.04	53.50	53.00	2.42	49.0	64.0	0.25
8	94	53.74	53.00	53.00	2.66	49.0	64.0	0.27
9	90	56.15	56.00	54.00	2.53	49.0	61.5	0.27
10	90	56.61	56.40	56.90	1.93	53.2	63.5	0.20
11	72	55.42	55.20	55.00	1.34	52.7	58.5	0.16
12	51	56.04	56.00	54.00	3.91	37.8	68.0	0.54
13	18	52.91	56.00	56.00	12.05	5.3	59.0	2.76
14	18	56.22	56.00	56.00	2.96	52.0	65.0	0.68
15	12	57.70	57.95	61.00	2.32	54.2	61.0	0.64

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	68.16	68.00	69.00	6.70	21.6	100.0	0.68
2	97	58.00	58.00	58.00	2.16	52.0	65.0	0.22
3	98	53.77	54.00	54.00	1.90	50.0	60.0	0.19
4	95	55.51	55.00	55.00	2.30	51.0	62.0	0.23
5	90	53.34	53.00	52.00	1.84	50.0	57.0	0.19
6	85	54.81	55.00	55.00	2.34	51.0	61.0	0.25
7	90	53.91	54.00	54.00	2.43	43.0	59.0	0.25
8	88	53.84	53.50	53.00	2.32	48.0	59.0	0.25
9	86	56.27	56.00	56.00	2.49	50.0	63.0	0.27
10	81	57.07	56.80	59.10	2.61	52.9	70.2	0.29
11	66	55.50	55.00	55.00	1.76	52.4	62.0	0.22
12	54	55.94	56.00	55.00	4.46	35.0	77.0	0.60
13	18	56.00	56.00	55.00	1.33	54.0	58.0	0.30
14	18	54.29	56.00	55.00	6.12	33.3	59.0	1.40
15	11	57.35	57.20	59.40	1.16	56.0	59.4	0.33

TABLE 6. RESULTS OF STATISTICAL ANALYSIS OF MEAN CORPUSCULAR HEMOGLOBIN IN SAMPLES FROM EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	21.33	21.10	21.10	1.73	19.4	33.8	0.18
2	100	19.77	19.60	19.90	0.84	16.8	22.5	0.08
3	98	18.77	18.60	18.60	0.95	15.9	22.1	0.10
4	95	18.79	18.30	18.30	1.63	17.1	28.6	0.17
5	94	18.58	18.50	18.00	0.73	17.1	20.8	0.08
6	93	18.90	18.90	19.20	0.64	16.9	21.0	0.07
7	92	18.94	19.00	19.20	0.74	16.3	21.1	0.08
8	94	18.99	18.90	18.90	0.79	17.4	22.9	0.08
9	90	19.38	19.40	19.70	0.76	17.9	21.7	0.08
10	90	20.29	20.20	19.90	0.54	19.1	21.5	0.06
11	72	19.80	19.60	19.50	1.00	18.2	26.2	0.12
12	51	20.91	20.30	20.40	5.08	18.9	56.0	0.70
13	18	20.86	20.80	22.20	0.92	19.3	22.2	0.21
14	18	20.86	20.75	22.20	0.92	19.3	22.2	0.21
15	12	21.71	21.45	21.00	1.08	20.2	23.3	0.30

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	21.37	21.00	20.70	1.76	19.1	31.6	0.18
2	97	19.79	19.70	19.90	0.71	18.5	22.1	0.07
3	98	18.79	18.60	18.30	0.92	17.2	21.9	0.09
4	95	18.59	18.30	18.20	1.08	16.9	21.9	0.11
5	90	18.57	18.45	18.00	0.84	17.4	23.0	0.09
6	85	18.97	18.80	18.70	0.70	17.4	20.8	0.08
7	90	18.97	18.80	18.80	0.85	15.4	21.1	0.09
8	88	19.03	18.90	19.00	0.79	17.7	21.2	0.08
9	86	19.48	19.40	19.70	1.32	17.9	30.0	0.14
10	81	20.56	20.30	20.70	1.20	19.2	28.7	0.13
11	66	19.74	19.60	19.40	0.68	18.3	22.4	0.08
12	54	20.68	20.30	20.40	4.88	10.6	54.0	0.66
13	18	21.11	21.20	21.90	0.66	19.9	22.0	0.15
14	18	22.99	21.35	21.90	7.76	19.9	54.0	1.78
15	11	21.87	21.80	22.60	0.86	20.7	23.6	0.25

TABLE 7. RESULTS OF STATISTICAL ANALYSIS OF MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATIONS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	30.53	30.80	30.70	3.66	0.0	36.3	0.39
2	100	34.52	34.05	33.20	2.15	27.9	41.0	0.21
3	98	35.04	35.10	35.70	1.81	30.3	39.6	0.18
4	95	33.59	33.00	33.80	2.24	29.3	45.2	0.23
5	94	34.58	34.55	34.70	1.27	31.7	38.2	0.13
6	93	34.44	34.50	34.90	1.27	30.8	36.8	0.13
7	92	35.23	35.20	36.80	1.51	30.7	39.7	0.16
8	94	35.36	35.65	37.00	1.54	31.6	38.1	0.16
9	90	34.56	34.60	35.80	1.54	30.9	38.6	0.16
10	90	35.84	35.90	36.60	1.14	31.4	37.9	0.12
11	72	35.65	35.65	35.60	0.95	33.4	37.9	0.11
12	51	35.17	35.80	35.70	4.22	11.4	38.2	0.59
13	18	39.66	37.40	37.40	6.59	34.5	57.7	1.51
14	18	37.48	37.40	37.40	0.78	35.8	39.5	0.18
15	12	37.59	37.20	37.20	0.84	36.6	39.3	0.23

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	33.62	31.10	31.30	28.56	13.0	311.3	2.88
2	97	34.34	33.90	34.20	2.05	31.1	40.7	0.21
3	98	35.08	35.10	35.70	2.37	21.1	44.4	0.24
4	95	33.55	33.20	33.90	1.73	30.3	38.1	0.18
5	90	34.77	34.75	35.30	1.21	31.1	39.1	0.13
6	85	34.64	34.70	35.30	1.52	30.4	37.7	0.16
7	90	35.28	35.50	36.80	1.57	31.5	38.9	0.16
8	88	35.39	35.60	35.50	1.62	30.1	38.3	0.17
9	86	34.40	34.50	34.20	1.47	30.6	38.1	0.16
10	81	35.79	35.90	36.20	1.14	32.3	38.8	0.13
11	66	35.69	35.60	35.80	1.13	33.5	39.9	0.14
12	54	35.62	35.95	35.80	2.78	19.3	39.9	0.37
13	18	36.91	37.20	38.20	2.19	31.0	39.5	0.50
14	18	36.64	37.30	38.20	4.23	20.1	39.5	0.97
15	11	38.09	38.10	38.10	0.89	36.6	40.1	0.26

TABLE 8. RESULTS OF STATISTICAL ANALYSIS OF LYMPHOCYTE COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	3.38	3.14	3.28	1.56	0.0	8.6	0.16
2	100	4.96	5.14	5.43	1.58	0.0	8.1	0.16
3	98	4.07	4.06	3.16	1.38	0.0	7.7	0.14
4	95	3.82	3.65	3.32	1.15	0.0	7.0	0.12
5	94	3.50	3.56	4.39	1.25	0.0	6.1	0.13
6	93	3.38	3.36	2.66	0.93	1.6	5.8	0.10
7	92	3.39	3.39	4.48	1.00	0.0	6.2	0.10
8	94	3.45	3.30	3.30	1.16	1.1	9.7	0.12
9	90	2.76	2.60	2.77	1.10	0.7	6.2	0.12
10	90	2.34	2.26	2.48	0.78	0.7	4.8	0.08
11	72	3.23	3.12	2.38	1.05	1.3	5.7	0.12
12	51	3.74	3.54	3.54	1.87	0.0	9.1	0.26
13	18	3.05	2.74	8.68	1.69	0.4	8.7	0.39
14	18	3.21	2.86	8.68	1.56	1.5	8.7	0.36
15	12	3.17	2.99	5.22	0.84	2.3	5.2	0.23

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	3.90	3.52	5.22	3.22	0.0	28.6	0.32
2	97	4.99	5.10	7.74	1.56	0.7	9.2	0.16
3	98	4.07	3.91	4.76	1.60	0.0	8.5	0.16
4	95	3.96	3.74	3.69	0.95	1.8	6.4	0.10
5	90	3.56	3.51	3.24	1.22	0.0	7.5	0.13
6	85	3.40	3.50	4.16	1.11	0.9	6.4	0.12
7	90	3.29	3.12	3.12	1.00	0.5	5.8	0.10
8	88	3.64	3.58	4.84	1.08	1.9	7.1	0.11
9	86	2.77	2.63	1.89	1.10	0.7	7.1	0.12
10	81	2.49	2.44	2.18	0.95	1.0	8.7	0.10
11	66	3.34	3.26	3.04	0.91	0.8	5.5	0.11
12	54	3.56	3.55	4.76	1.67	0.0	11.3	0.23
13	18	2.91	2.91	3.89	0.58	1.7	3.9	0.13
14	18	3.33	3.07	10.07	1.77	1.7	10.0	0.41
15	11	3.48	3.60	5.46	1.20	1.5	5.5	0.34

TABLE 9. RESULTS OF STATISTICAL ANALYSIS OF NEUTROPHIL COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	1.24	0.92	0.61	1.06	0.0	5.2	0.11
2	100	0.78	0.65	0.99	0.58	0.0	3.2	0.06
3	98	0.95	0.81	1.17	0.65	0.0	3.2	0.06
4	95	0.84	0.63	0.72	0.93	0.0	8.4	0.10
5	94	1.27	1.12	0.70	0.87	0.0	4.1	0.09
6	93	1.26	1.22	1.61	0.55	0.2	2.6	0.06
7	92	0.94	0.77	0.43	0.60	0.0	3.2	0.06
8	94	1.25	1.10	1.19	0.81	0.2	5.7	0.08
9	90	1.25	1.25	1.56	0.59	0.4	3.9	0.06
10	90	1.92	1.63	3.13	1.19	0.4	8.9	0.12
11	72	1.38	1.04	1.49	1.52	0.2	11.7	0.18
12	51	2.14	1.84	1.80	1.33	0.0	6.2	0.18
13	18	1.75	1.50	3.86	0.97	0.3	3.9	0.22
14	18	1.62	1.43	3.22	0.82	0.3	3.2	0.19
15	12	2.61	1.71	1.13	2.00	0.9	6.7	0.55

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	1.41	1.06	0.54	1.57	0.0	13.5	0.16
2	97	0.95	0.83	1.07	0.68	0.1	3.3	0.07
3	98	1.13	0.95	1.89	0.63	0.0	2.9	0.06
4	95	0.84	0.72	0.43	0.59	0.0	3.5	0.06
5	90	1.18	1.01	0.76	0.72	0.0	3.0	0.08
6	85	1.28	1.13	1.03	0.64	0.0	3.3	0.07
7	90	0.96	0.86	1.12	0.50	0.2	2.5	0.05
8	88	1.13	0.93	0.92	0.70	0.2	3.2	0.07
9	86	1.30	1.25	1.40	0.66	0.2	3.8	0.07
10	81	1.78	1.72	1.72	0.72	0.2	4.1	0.08
11	66	1.05	0.93	0.76	0.62	0.3	3.3	0.08
12	54	1.88	1.95	2.77	0.88	0.3	3.8	0.12
13	18	1.97	1.48	1.97	1.70	0.8	8.2	0.39
14	18	2.25	1.48	1.97	2.01	1.0	8.2	0.46
15	11	1.72	1.60	4.10	1.10	0.0	4.1	0.31

TABLE 10. RESULTS OF STATISTICAL ANALYSIS OF EOSINOPHIL COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	0.03	0.00	0.06	0.05	0.0	0.3	0.01
2	100	0.06	0.06	0.06	0.07	0.0	0.3	0.01
3	98	0.07	0.05	0.05	0.07	0.0	0.3	0.01
4	95	0.09	0.07	0.05	0.07	0.0	0.3	0.01
5	94	0.07	0.05	0.05	0.08	0.0	0.4	0.01
6	93	0.08	0.06	0.11	0.09	0.0	0.6	0.01
7	92	0.09	0.08	0.05	0.08	0.0	0.4	0.01
8	94	0.13	0.11	0.11	0.13	0.0	0.6	0.01
9	90	0.06	0.04	0.04	0.07	0.0	0.3	0.01
10	90	0.06	0.05	0.10	0.08	0.0	0.4	0.01
11	72	0.09	0.06	0.07	0.09	0.0	0.5	0.01
12	51	0.11	0.07	0.05	0.14	0.0	0.7	0.02
13	18	0.17	0.10	0.10	0.20	0.0	0.8	0.04
14	18	0.18	0.10	0.10	0.19	0.0	0.8	0.04
15	12	0.07	0.06	0.24	0.08	0.0	0.2	0.02

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	0.03	0.00	0.04	0.05	0.0	0.2	0.00
2	97	0.10	0.07	0.15	0.10	0.0	0.4	0.01
3	98	0.09	0.07	0.07	0.19	0.0	0.5	0.01
4	95	0.08	0.07	0.04	0.07	0.0	0.3	0.01
5	90	0.09	0.07	0.04	0.08	0.0	0.4	0.01
6	85	0.07	0.07	0.04	0.07	0.0	0.3	0.01
7	90	0.10	0.09	0.07	0.08	0.0	0.4	0.01
8	88	0.14	0.10	0.04	0.13	0.0	0.5	0.01
9	86	0.08	0.07	0.08	0.07	0.0	0.4	0.01
10	81	0.06	0.04	0.09	0.07	0.0	0.3	0.01
11	66	0.09	0.08	0.08	0.08	0.0	0.5	0.01
12	54	0.09	0.07	0.07	0.10	0.0	0.5	0.01
13	18	0.18	0.16	0.16	0.15	0.0	0.5	0.03
14	18	0.19	0.17	0.17	0.14	0.0	0.5	0.03
15	11	0.14	0.16	0.16	0.11	0.0	0.3	0.03

TABLE 11. RESULTS OF STATISTICAL ANALYSIS OF MONOCYTE COUNTS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	89	0.05	0.04	0.04	0.06	0.0	0.3	0.01
2	100	0.13	0.12	0.07	0.12	0.0	0.6	0.01
3	98	0.10	0.06	0.05	0.10	0.0	0.5	0.01
4	95	0.05	0.00	0.06	0.07	0.0	0.3	0.01
5	94	0.04	0.04	0.05	0.05	0.0	0.3	0.01
6	93	0.06	0.05	0.05	0.06	0.0	0.2	0.01
7	92	0.08	0.05	0.09	0.11	0.0	0.7	0.01
8	94	0.11	0.05	0.05	0.12	0.0	0.5	0.01
9	90	0.90	0.08	0.08	0.08	0.0	0.3	0.01
10	90	0.32	0.29	0.29	0.19	0.0	0.8	0.02
11	72	0.08	0.05	0.05	0.12	0.0	0.7	0.01
12	51	0.34	0.08	0.16	1.29	0.0	8.5	0.18
13	18	0.12	0.09	0.09	0.13	0.0	0.5	0.03
14	18	0.12	0.09	0.09	0.12	0.0	0.4	0.03
15	12	0.18	0.10	0.73	0.25	0.0	0.7	0.07

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	0.06	0.04	0.06	0.07	0.0	0.2	0.01
2	97	0.16	0.11	0.07	0.17	0.0	1.0	0.02
3	98	0.10	0.08	0.07	0.08	0.0	0.5	0.01
4	95	0.05	0.03	0.04	0.07	0.0	0.4	0.01
5	90	0.04	0.00	0.06	0.05	0.0	0.2	0.01
6	85	0.06	0.05	0.05	0.06	0.0	0.2	0.01
7	90	0.07	0.05	0.05	0.08	0.0	0.3	0.01
8	88	0.10	0.06	0.17	0.11	0.0	0.4	0.01
9	86	0.08	0.05	0.04	0.08	0.0	0.4	0.01
10	81	0.32	0.27	0.27	0.21	0.0	1.0	0.02
11	66	0.08	0.06	0.11	0.10	0.0	0.5	0.01
12	54	0.40	0.07	0.07	1.20	0.0	6.6	0.16
13	18	0.14	0.06	0.05	0.16	0.0	0.6	0.04
14	18	0.14	0.09	0.05	0.16	0.0	0.6	0.04
15	11	0.06	0.00	0.29	0.11	0.0	0.3	0.03

Discussion

When a pathophysiological approach is used in evaluating hematological data, interpretations are seldom individually diagnostic but are invaluable as an adjunct to the health profile. The major interpretations of hematological test results pertain to the presence or absence of systemic stress, inflammatory disease, bone marrow disease, and neoplasia. This type of information helps in formulating a presumptive or final diagnosis and in monitoring physiological or pathological abnormalities.

Hematological parameters reflect the erythropoietic activity of the bone marrow. There was no significant stimulation or impairment of either total erythrocyte numbers, MCV, MCH, or MCHC in exposed rats compared with in the sham-exposed rats. These findings indicate that the 25-month exposure had no significant effect either on the bone marrow and erythropoietic cells or on the juxtaglomerular apparatus of the kidney and its production of erythropoietin. Erythropoietin is a circulating glycoprotein hormone that stimulates the bone marrow to produce erythrocytes, apparently by promoting the conversion of primitive stem cells into erythroid elements. The RFR exposure did not appear to have any effect on the intracellular metabolism of the erythropoietic cells necessary for normal cell production and hemoglobin synthesis.

The quality control of the hematological data maintained precision of results, which in turn enabled detection of subtle decreases in total lymphocyte counts with age. The increases in neutrophil, monocyte, and eosinophil counts with age reflect the increasing incidence of spontaneous tumors and degenerative diseases and their release of chemotactic agents which increase the levels of these granulocytic cells. These changes in differential counts did not significantly differ between exposed and sham-exposed populations.

The significant decrement in eosinophil count during session 2 is interesting in light of the significant elevation of corticosterone during this session and the eosinopenic effect of elevated corticosteroids. Additional correlation of these findings and discussion of specific lesions and other parameters with the hematological indices will be presented in Volume 9 of this series.

SERUM CHEMISTRY

Clinical pathology involves measuring chemical constituents in biologic material and interpreting the results in the pursuit of a biochemical understanding of pathogenesis and its responsiveness to various therapeutic or experimental maneuvers. A complete serum-chemistry panel was performed on samples obtained from a baseline group at the start of the experiment and from samples taken every 6 weeks for nine sampling sessions during the first year and then every 12 weeks until the end of the 25-month exposure period.

In sampling sessions in which corticosterone samples were taken, the volume of serum obtained for blood-chemistry evaluation was generally insufficient due to the large volume of blood (2 ml) required for the corticosterone sample. For these sessions, most but not all of the serum samples required dilution prior to analysis. Since it was known that serum dilution would affect the assayed results of some parameters, before the experiment a serum pool was prepared from which samples could be withdrawn throughout the study for use as a control serum. Run concurrently with each sampling were 40 control samplings: 20 were run undiluted, and 20 were assayed after dilution to levels representative of dilution factors used on the actual serum obtained from the animals under experimental treatment. Comparison studies of whole serum and diluted serum were performed on the SMAC analyzer. Pooled rat serum was also used for evaluating within-run and between-run variation and the effect of dilution on linearity. Diluted samples with results outside the no-significant-nonlinearity range were eliminated from the data. A few selected tests with results within the no-significant-nonlinearity range required a "constant factor" to standardize the precision of their results.

The analytic bias (accuracy) of the serum chemistry results was evaluated by means of a quality-control chart used for comparing the analytical results obtained within a test run with normal and abnormal reference samples, assayed standards, and an in-house reference pool. When these controls were not within acceptable limits, the analytical test results were not considered valid, and the source of analytical error was found and the error corrected. The action limits and the warning limits of the control serum were calculated from the determined mean (X) and

coefficient of variation (C.V.) of the tests. The action limit, were X ± 3 C.V., subject to a maximum X ± 20% for enzymes and X ± 15% for nonenzymes. The warning limits were X ± 2 C.V. No warning limits existed when X ± 2 C.V. was close to the action limit, such as the creatinine test with 7% C.V. (Mia, 1977).

The within-run random analytic variation can be seen by a comparison of the mean, variance, standard deviation, and coefficient of variation. Since the magnitude of the standard deviation is often directly related to the magnitude of the mean, the term "coefficient of variation" has become the most common way of representing the random analytic variation (Statland, 1979).

Methods

Techniques for evaluating rat serum have been developed for the Technicon SMAC computer-controlled biochemical analyzer. Many samples can be analyzed in 1 day, with reliable precision and minimal within-run and run-to-run random analytic variation, by chemistry methodologies compatible with rat serum.

Because the amount of serum available from each rat was limited, especially during blood-sampling sessions when samples were drawn for corticosterone and T_4 assays, dilution techniques were developed to maximize the amounts for evaluation. The use of dilutions was reliable and valid, from the following observations:

1. No mean values for any parameter varied dramatically from values for sessions involving data correction due to dilution (sessions 1, 2, 4, 6, 8, and 10) and for sessions in which nondiluted samples were available (sessions 3, 5, 7, 9, and 11-15).

2. Mean values obtained for all parameters were within the range of standard values in the literature.

The following determinations were obtained on each serum sample:

1. Glucose
2. Blood urea nitrogen (BUN)
3. Creatinine

4. Sodium
5. Potassium
6. Chloride
7. Carbon dioxide
8. Uric acid
9. Total bilirubin
10. Direct bilirubin
11. Calcium
12. Phosphorus
13. Alkaline phosphatase
14. LDH
15. SGOT
16. SGPT
17. Cholesterol
18. Triglycerides
19. Total protein
20. Albumin
21. Globulin

Multiple pathophysiological interrelationships can significantly affect each of these serum chemistry determinations, some of which are discussed in connection with the individual chemistry methodologies presented in following sections.

Glucose

Increased blood glucose levels (hyperglycemia) can be caused by diabetes mellitus, seizures, pancreatitis, pheochromocytoma (neoplasm), treatment with drugs (corticosteroids, dextrose, epinephrine), and transiently by excitement or stress. Decreased blood glucose levels (hypoglycemia) can be related to artifact, severe infection, hepatic insufficiency, glycogen storage disease, malabsorption or starvation, insulinoma (neoplasm), generalized or large neoplasm, adrenal insufficiency, and renal glucosuria. The bound-hexokinase method as reported by Leon et al. (1977) was used for determining glucose in the serum.

Blood Urea Nitrogen

Increased BUN levels are associated with renal insufficiency, obstructive uropathy, gastrointestinal bleeding, and other causes of prerenal uremia. Reduced levels of BUN may result from severe hepatopathy, low protein intake, fluid therapy, malabsorption, and hepatic venous shunts. Creatinine tests and urinalysis are used to further evaluate an abnormal BUN. The method used for direct determination of urea was the diacetyl-monoxime method originally described by Marsh et al. (1965).

Creatinine

The same conditions that elevate the BUN will elevate creatinine; however, the creatinine level is not affected by dietary protein, catabolism, or exercise. Creatinine is increased significantly by skeletal muscle necrosis or atrophy associated with trauma and the rapidly progressing muscular dystrophies, and is associated with hyperthyroidism and diabetic acidosis. Low levels of creatinine are not clinically significant. The method used for determining creatinine is based on its reaction with saturated picric acid in an alkaline medium as described by Chasson et al. (1961).

Sodium

Increased sodium can result from dehydration or excessive salt intake with restricted water consumption and rarely occurs in hyperadrenocorticism. Decreased sodium occurs with hypoadrenocorticism, renal disease, and prolonged diarrhea. A direct potentiometric procedure using a sodium ion-selective glass electrode, based on the original work of Rao et al. (1973), was used to quantitatively measure sodium in serum.

Potassium

Serum potassium levels increase in renal disease hypoadrenocorticism, acidosis, shock, and circulatory failure. The levels decrease in hyperadrenocorticism, metabolic alkalosis, prolonged vomiting, and/or diarrhea. The direct potentiometric procedure for quantitatively measuring potassium by means of an ion-selective electrode of the type developed by Pioda et al. (1970) was used.

Chloride

Increased serum chloride can result from metabolic acidosis, respiratory alkalosis (hyperventilation), decreased excretion (urinary tract obstruction, nephrosis), excessive salt intake, limited water intake, and hyperadrenocorticism. The levels decrease with metabolic alkalosis, respiratory acidosis (hypoventilation), persistent vomiting (high intestinal obstruction), chronic renal disease, and hypoadrenocorticism. The colorimetric procedure for quantitatively measuring chloride as reported by Morgenstern et al. (1973) was used.

Total Carbon Dioxide

Total carbon dioxide ($T.CO_2$) is a measure of the amount of dissolved carbon dioxide (CO_2), carbonic acid (H_2CO_3), and biocarbonate ions (HCO_3) in the serum. Increased levels of $T.CO_2$ may reflect metabolic acidosis, respiratory alkalosis, or be a sampling artifact. In the method used, CO_2 released by acid is absorbed by an alkaline buffer solution containing phenophthalein; the decrease in red color is proportional to the CO_2 content of the sample. This principle and the development of a silicon rubber dialyzer membrane for the CO_2 gas separation was reported by Skaggs and Hochstrasser (1964).

Uric Acid

In general, normal rat serum has a lower level of uric acid than does human serum. In most mammals, purines are metabolized to uric acid and then, by the enzyme uricase, to allantoin in the liver. In rats, uric acid levels are increased only when the liver cells are damaged, with incomplete conversion of uric acid to allantoin resulting in elevated uric acid in the blood and urine. The uric acid colorimetric method used is based on the reduction of a phosphotungstate complex to a phosphotungstite complex. A dialyzer is used to obtain a protein-free solution as described by Musser and Ortigoza (1966).

Total Bilirubin and Direct Bilirubin

Increased total bilirubin levels are generally caused by hemolysis, heptocellular disease (infection, neoplasm), cholestatis disease, extrahepatic biliary tract obstruction, and resolving internal hemorrhage. The total levels are decreased in cases of bone-marrow-depression anemia. The direct and indirect bilirubin levels help differentiate hemolytic, obstructive, and hepatocellular origins of the elevated bilirubin. Urine bilirubin and urobilinogen are follow-up tests for abnormal bilirubin findings. The colorimetric procedure for direct bilirubin measurement follows the automated method described by Gambino and Schreiber (1965).

Total Calcium

Increased serum calcium levels are associated with pseudohyperparathyroidism (usually lymphosarcoma), primary hyperparathyroidism (neoplasms), secondary hyperparathyroidism (renal), metastic neoplasia (osseous metastasis), multiple myeloma (bone lysis), increase in dietary vitamin D or calcium, and lipemia. Decreased levels of serum calcium can result from

hypoparathyroidism, renal insufficiency, steroid elevation, acute hypoalbuminemia, hemolysis, and young age.

A metal-complexing dye (cresolphthalein complexone) that can bind calcium ions in an alkaline medium is used for determination, based on the work of Gitelman (1967). The product of this interaction is a pink-colored calcium dye complex that can be quantitated. This method incorporates the use of 8-hydroxyquinoline, which virtually eliminates magnesium interferences.

Phosphorus

Increases in serum phosphorus may be the result of renal disease or failure, pseudohyperparathyroidism, obstructive uropathy, shock, or dehydration, or may be associated with young age. Decreased levels may be caused by primary hyperparathyroidism, severe liver disease, low protein intake, or malabsorption. The method for determining inorganic phosphorus, described by Amador and Urban (1972), is based on the fact that an unreduced phosphomolybdate complex absorbs ultraviolet light.

Alkaline Phosphatase

Elevated alkaline phosphatase levels occur in young animals, hepatocellular disease, lymphosarcoma, bone genesis and resorption, chronic skin lesion, renal disease, hyperadrenocorticism, intestinal mucosal disorders, and treatment with corticosteroids. Decreased levels have little or no clinical significance. At room temperature, serum values may increase 5 to 30% in 12 h.

A method reported by Morgenstern et al. (1965), based on the enzymatic hydrolysis of p-nitrophenyl phosphate, which produces a yellow color, is used for determining alkaline phosphatase. After incubation, a dialyzer, placed in the reaction stream, separates the p-nitrophenol from bilirubin interference, thereby eliminating the need for blank correction.

Lactate Dehydrogenase

Elevated levels of LDH, an intracellular enzyme, can usually be traced to young age, hemolysis, and tissue necrosis. Decreased levels of LDH have no apparent clinical significance.

The LDH kinetic procedure used was reported by Morgenstern et al. (1973). The LDH level is based on the reduction of NAD to NADH, which is proportional to the amount of LDH enzymatic activity.

Serum Glutamic-Oxaloacetic Transaminase
(syn: asparate amino transferase-ASAT)

Increased serum levels of SGOT may be the result of hepatic disease, muscle damage, myocardial necrosis, pancreatic necrosis, and hemolysis. Reduced SGOT levels have no clinical significance. Creatinine phosphokinase (CPK) is a suggested follow-up test for investigating an abnormal SGOT finding. The kinetic procedure reported by Kessler et al. (1975) is used for determining SGOT.

Serum Glutamic-Pyruvate Transaminase
(syn: alanine amino transferase-ALAT)

An elevated SGPT is usually associated with liver damage (primary hepatocellular necrosis or secondary hepatocellular damage). A reduced SGPT has no clinical significance. The kinetic procedure reported by Kessler et al. (1975) is used.

Cholesterol

Serum cholesterol levels are increased in hypothyroidism, diabetes mellitus, pancreatitis, liver disease (hepatocellular disease, biliary obstruction), renal disease (nephrosis and occasionally nephritis), and

hyperadrenocorticism. Decreased levels may be seen in animals on a low saturated-fat diet.

An enzymatic method is used to determine serum cholesterol concentration. In this method cholesterol esterase is used to hydrolyze the cholesterol esters in serum to free cholesterol. The free cholesterol is then oxidized to produce hydrogen peroxide, which is used to form a quinonimine dye. The concentration of the dye is measured colorimetrically and is directly proportional to the cholesterol content in the serum sample. This procedure was reported by Leon and Stasiw (1976).

Triglycerides

In man both cholesterol and triglycerides have been identified as risk factors related to atherosclerotic disease. Their levels can vary independently, so hyperlipidemia evaluation should include determinations for both of these lipids. The method used is based on the work of Bucolo and David (1973). Prior purification of the serum sample is not required since the enzyme lipase is specific for triglycerides. The hyperlipidemias can be an inherited trait or they can be secondary to a variety of disorders, including diabetes mellitus, nephrosis, bilary obstruction, and metabolic disorders associated with endocrine disturbances (thyroid).

Total Protein

The causes of increased levels of serum total protein include dehydration, shock, polyclonal gammopathy (seen in aging and infection--a response to antigens), and monoclonal gammopathy (lymphosarcoma, multiple myeloma, plasmatoma). Decreased total protein levels may result from blood or serum loss, protein-losing enteropathies, malabsorption, hepatopathies, nephropathies, deficient intake, and neoplasms. A serum protein electrophoresis is suggested as a follow-up test for investigating abnormal total-protein values.

The total-protein method is based on the work of Skaggs and Hochstrasser (1964). This is a biuret reagent procedure in which sodium

potassium tartrate is the complexing agent and potassium iodine is added to the biuret reagent to prevent autoreduction. The procedure is acceptable for the rat. Some other protein methods are dye-binding procedures, but these procedures are dependent on the charges on the protein and thus vary with the species.

Albumin

Increased serum albumin (hyperalbuminemia) is rarely observed except where associated with dehydration. Decreased albumin levels are usually caused by glomerulopathies, protein-losing enteropathy, malabsorption, hepatopathies, severe blood or serum loss, or negative nitrogen balance, and are associated with monoclonal and polyclonal gammopathies. Serum protein electrophoresis and urine protein determinations are recommended as follow-up tests for investigating abnormal albumin levels.

The albumin is measured by the bromcresol-green method described by Doumas et al. (1971). This method does not require the correction factor that is needed when the HABA (2-(p-hydroxyphenylazo)-benzoic acid) dye-binding procedure is used and human serum is used as the standard.

Globulin

Increased globulin levels are attributed to immune-mediated diseases, active hepatopathy, chronic infections with immune response, monoclonal gammopathies, polyclonal gammopathies, and amyloidosis. Serum globulin is computed indirectly by the SMAC by subtracting the albumin from the total protein. A direct globulin value is obtained from the protein electrophoresis.

Results

The initial baseline data on serum chemistry (blood sampling 1) and the data from nine subsequent 6-week intervals (samplings 2-10) and

five 12-week intervals (11-15) are presented in Figs. 15 through 34. The bar graphs represent mean values and standard errors for exposed and sham-exposed animals based on the combined values for all animals in each treatment group.

The data presented represent all data collected and incorporate the simple-integer correction factors required by the specific dilution used on any particular blood sample, but no correction for the minor nonproportional errors resulting from dilution. During statistical analysis, all data from a sample were dropped if the sample had been diluted at a factor of 1:2 or greater. This procedure was adopted after a preliminary analysis indicated that dilutions at this level significantly affected the accuracy of determinations. This procedure was not followed for the first blood sampling, during which nearly all samples were diluted at 1:2; therefore, the mean of neither exposed nor sham-exposed populations was differentially affected. Table 12 presents a sample of the frequency of dilution-factor use by sampling session and treatment condition. The table also presents a tally of animals from which no blood was drawn, data were missing owing to failure of the chemistry analyzer, the quantity was insufficient for determination, and the number of dead animals at the time of sampling.

Results of the statistical analysis of the serum chemistry data are presented for each parameter in Tables 13 through 32, including the mean, maximum and minimum, standard deviation, and standard error.

Examination of the data revealed that whereas some distributions were skewed, most were near normal; therefore, no transformations of the data were made prior to analysis. Inspection of the correlation matrices for all parameters indicated that, except for cholesterol and triglycerides, run-to-run correlations were uniformly low. For both cholesterol and triglycerides, adjacent-session correlations were relatively high.

Multivariate analysis of the data was deemed unnecessary; however, individual t-tests were made for all mean comparisons. Among the 300 individual pairwise t-test comparisons made for all 20 parameters for 15 sessions, only a few isolated comparisons were significant at the .05 level and no coherent pattern was discernible.

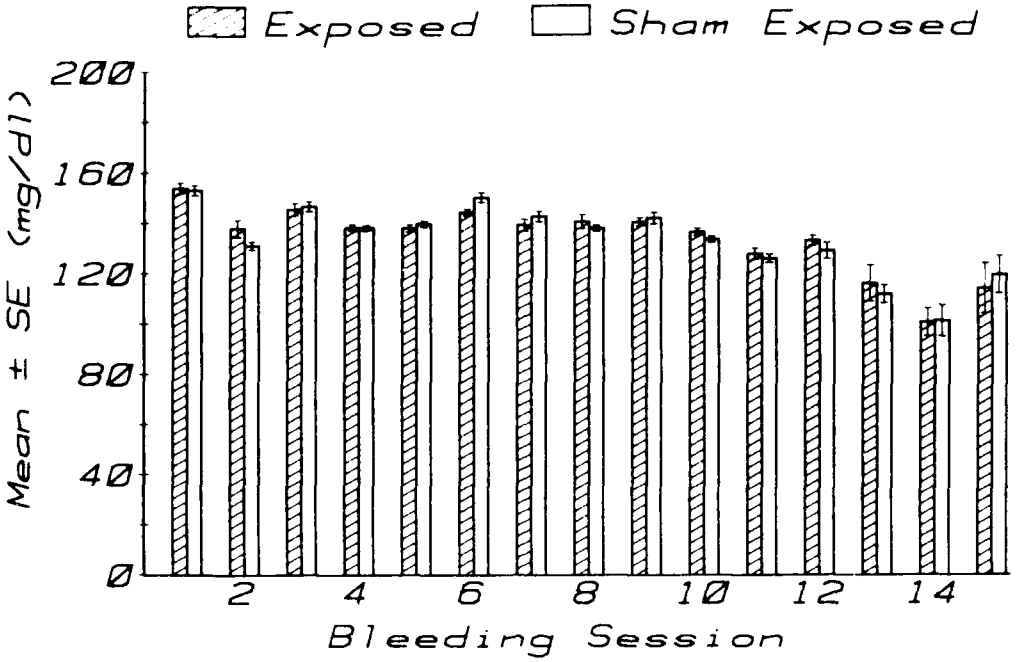

Fig. 15. Comparison of data on serum GLUCOSE for exposed and sham-exposed animals for 15 sampling sessions.

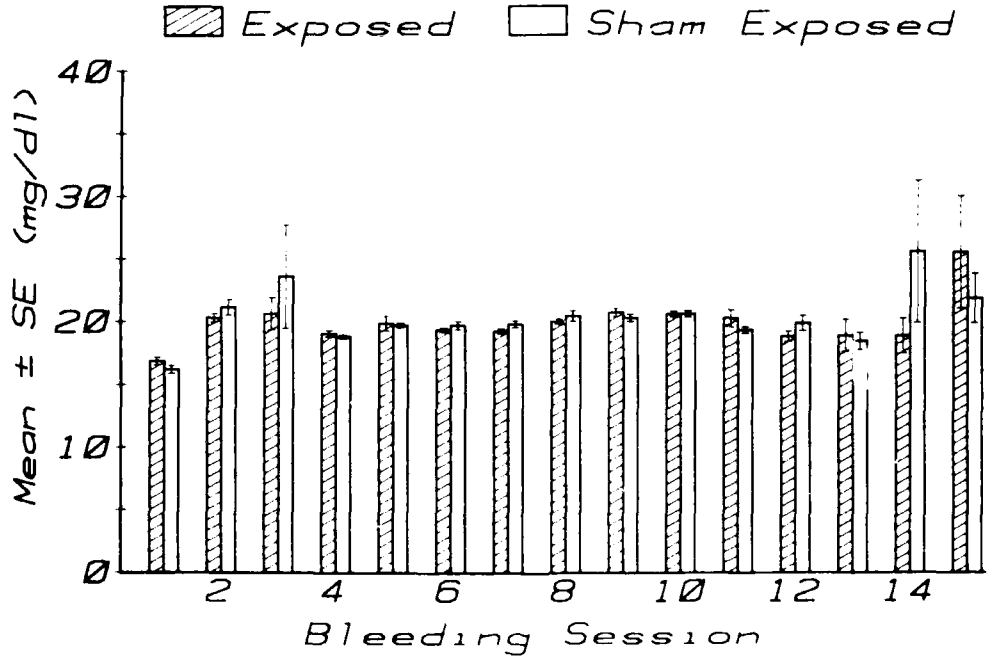

Fig. 16. Comparison of data on serum BLOOD UREA NITROGEN for exposed and sham-exposed animals for 15 sampling sessions.

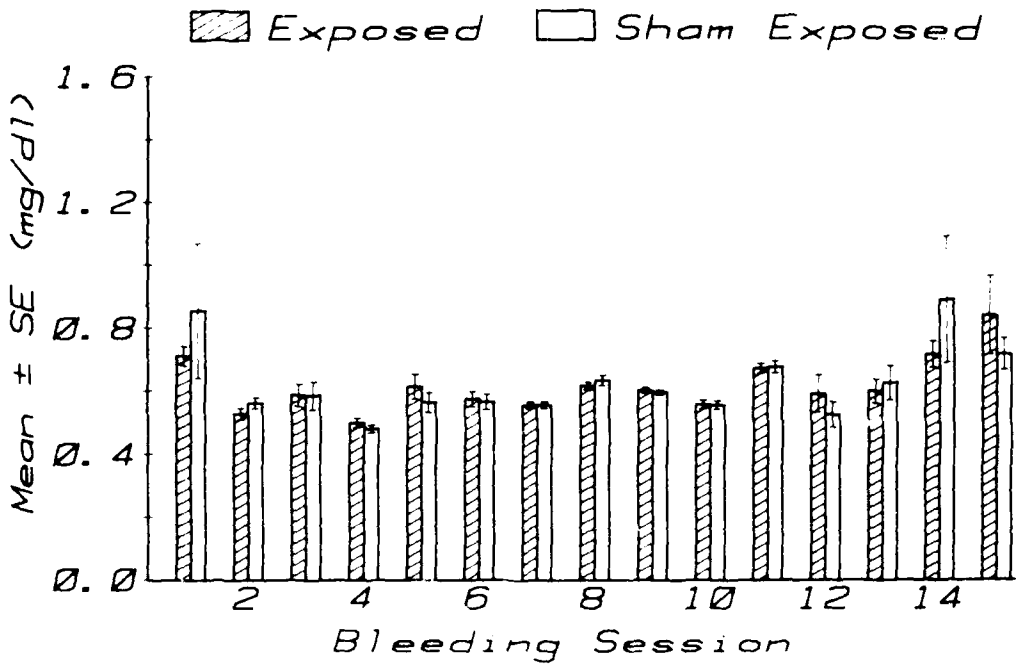

FIG. 17. Comparison of data on serum CREATININE for exposed and sham-exposed animals for 15 sampling sessions.

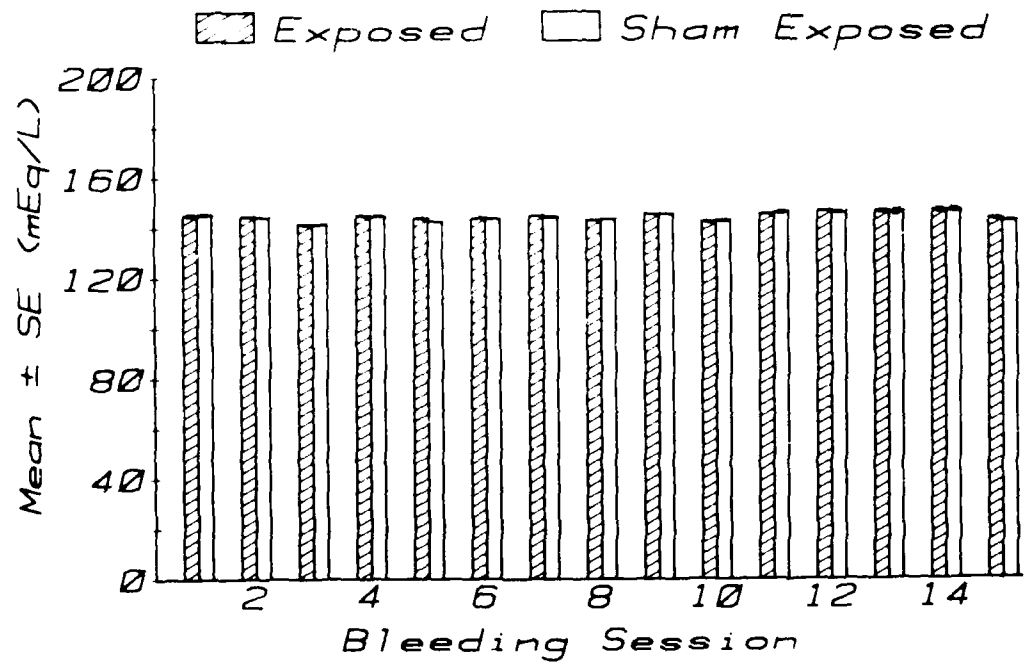

FIG. 18. Comparison of data on serum SODIUM for exposed and sham-exposed animals for 15 sampling sessions.

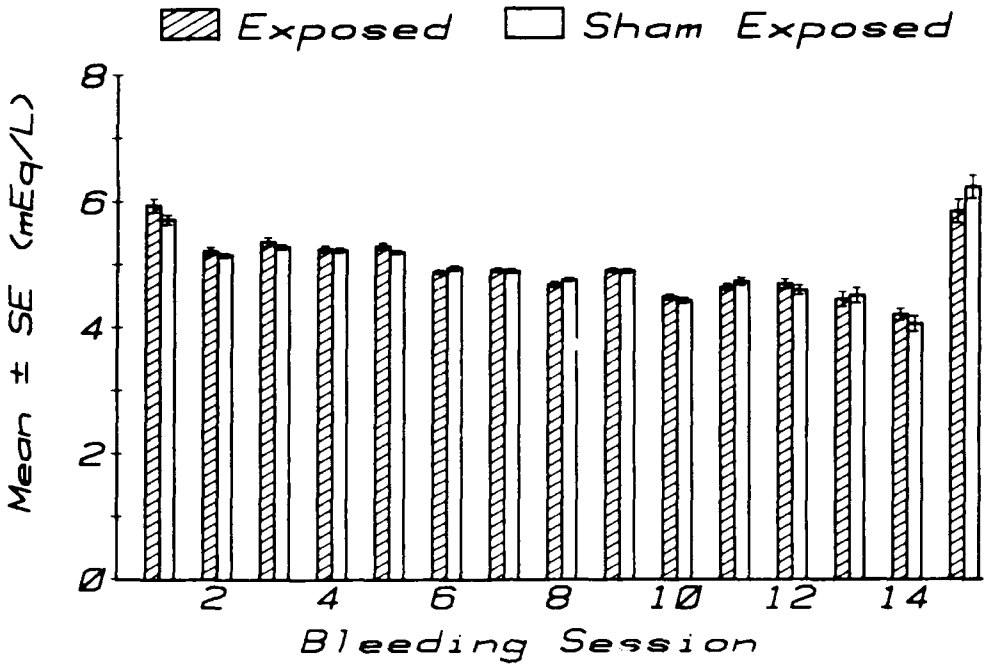

Fig. 19. Comparison of data on serum POTASSIUM for exposed and sham-exposed animals for 15 sampling sessions.

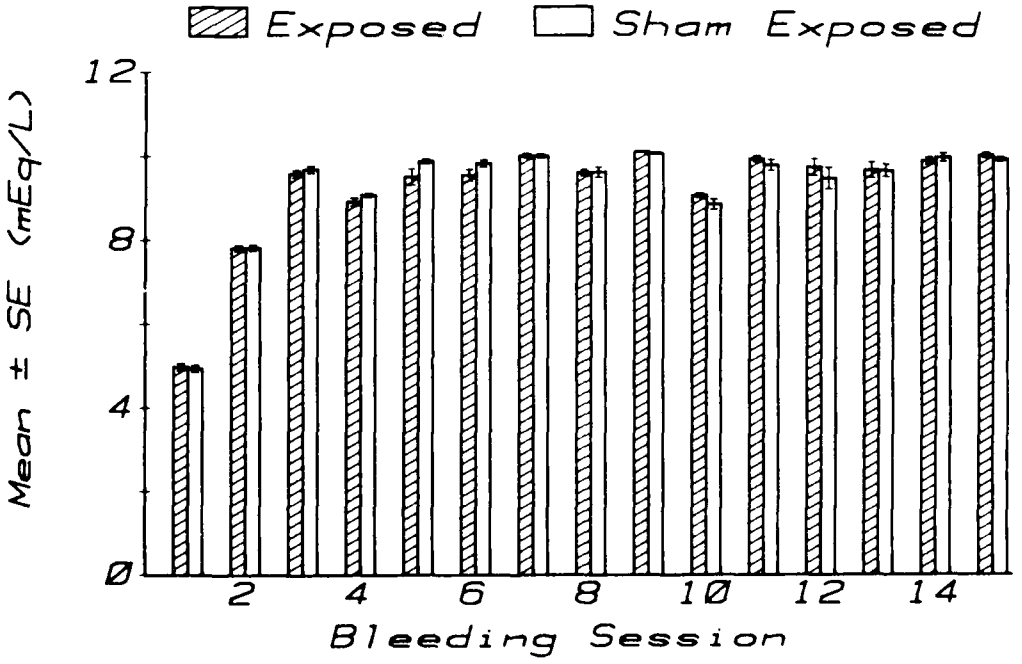

Fig. 20. Comparison of data on serum CHLORIDE for exposed and sham-exposed animals for 15 sampling sessions.

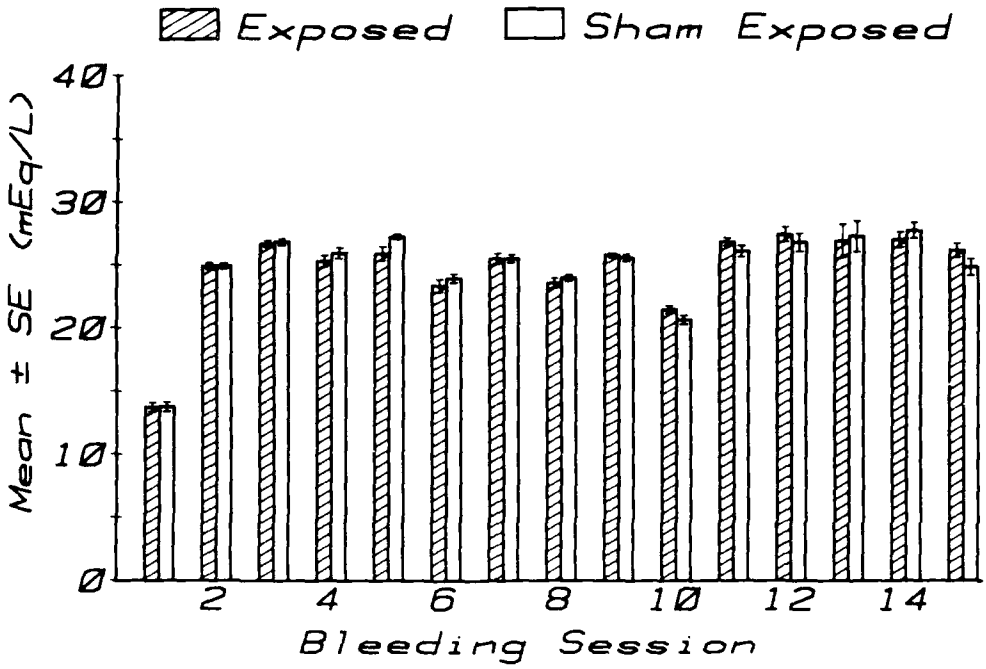

Fig. 21. Comparison of data on serum CARBON DIOXIDE for exposed and sham-exposed animals for 15 sampling sessions.

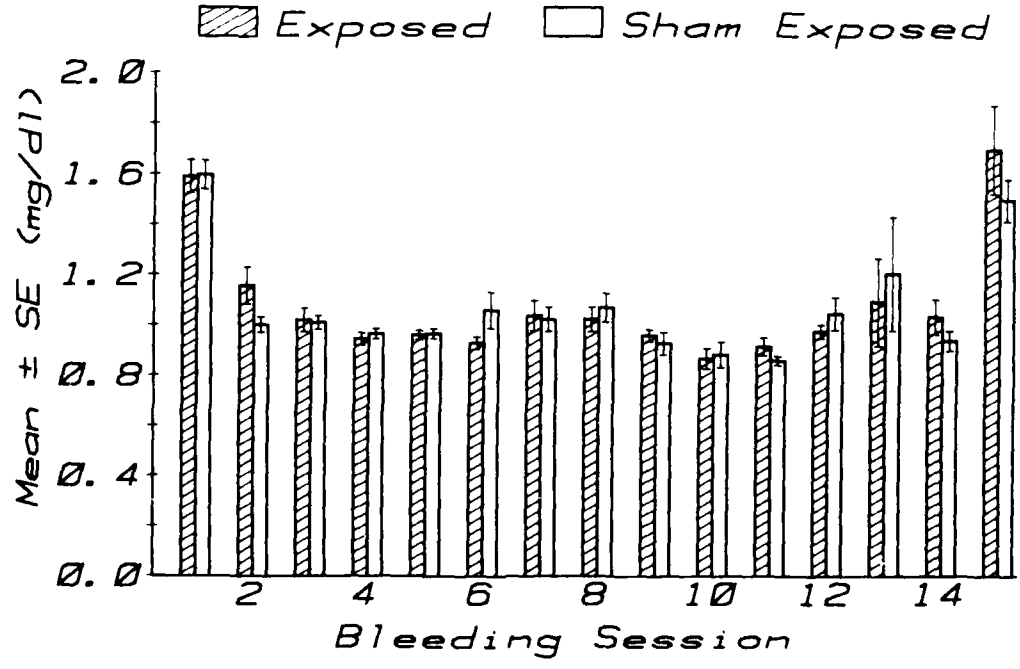

Fig. 22. Comparison of data on serum URIC ACID for exposed and sham-exposed animals for 15 sampling sessions.

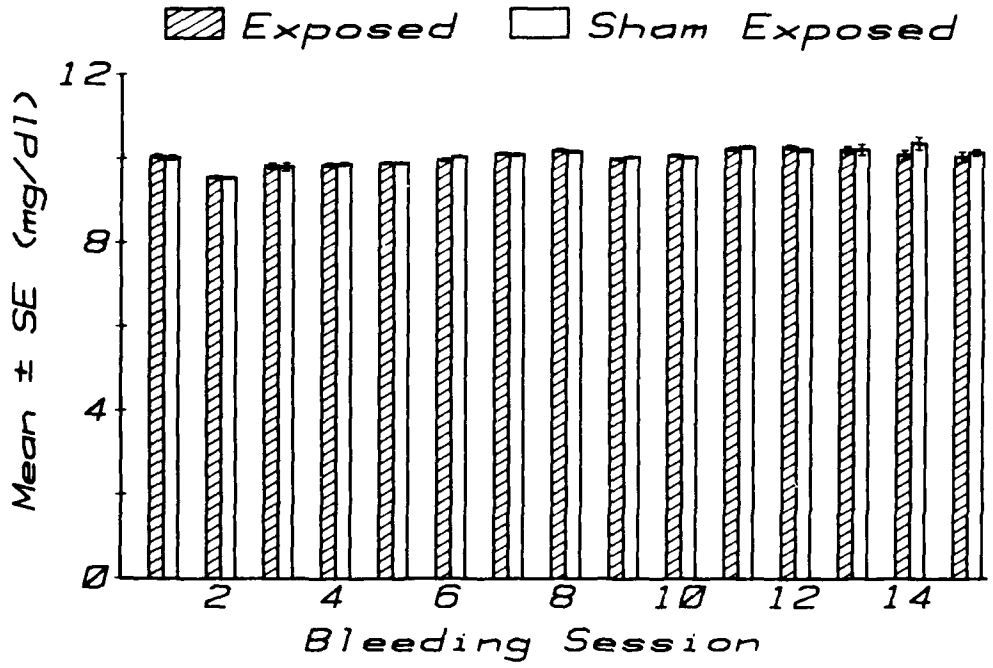

Fig. 23. Comparison of data on serum CALCIUM for exposed and sham-exposed animals for 15 sampling sessions.

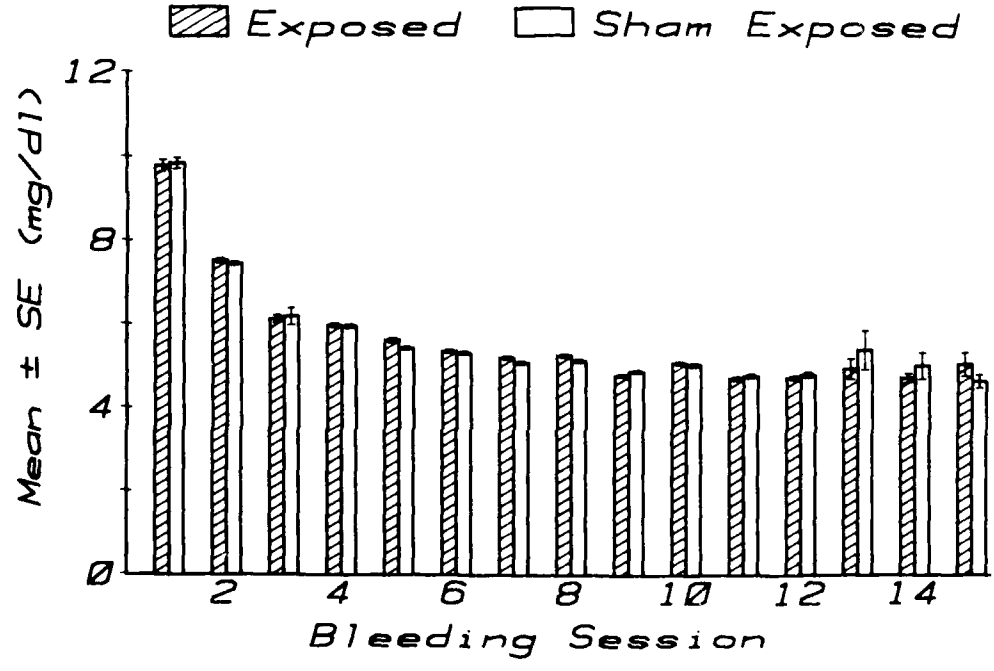

Fig. 24. Comparison of data on serum PHOSPHORUS for exposed and sham-exposed animals for 15 sampling sessions.

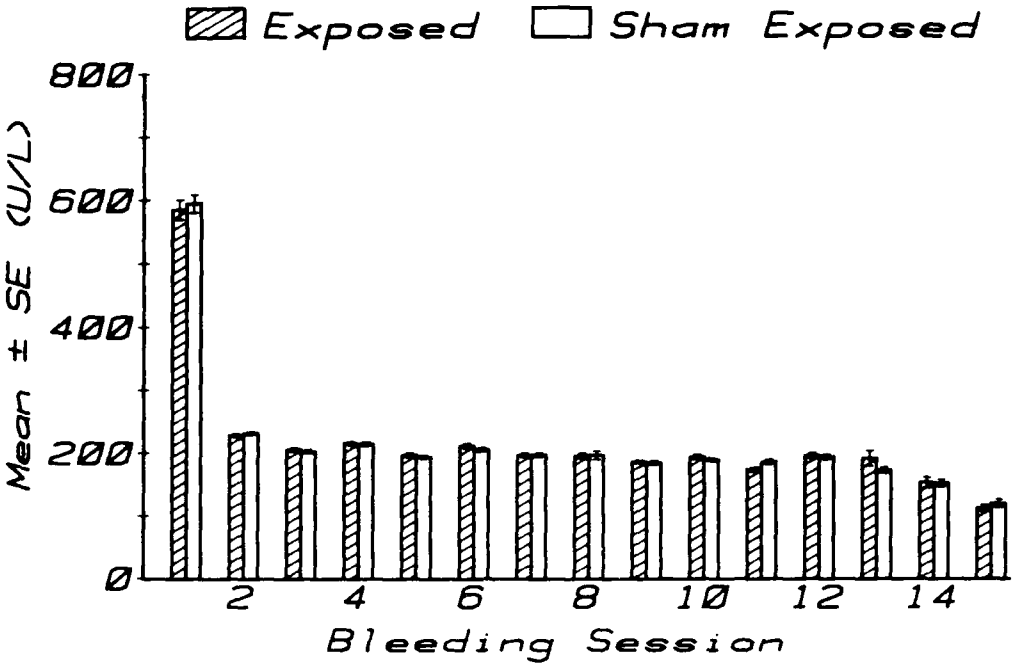

Fig. 25. Comparison of data on serum ALKALINE PHOSPHATASE for exposed and sham-exposed animals for 15 sampling sessions.

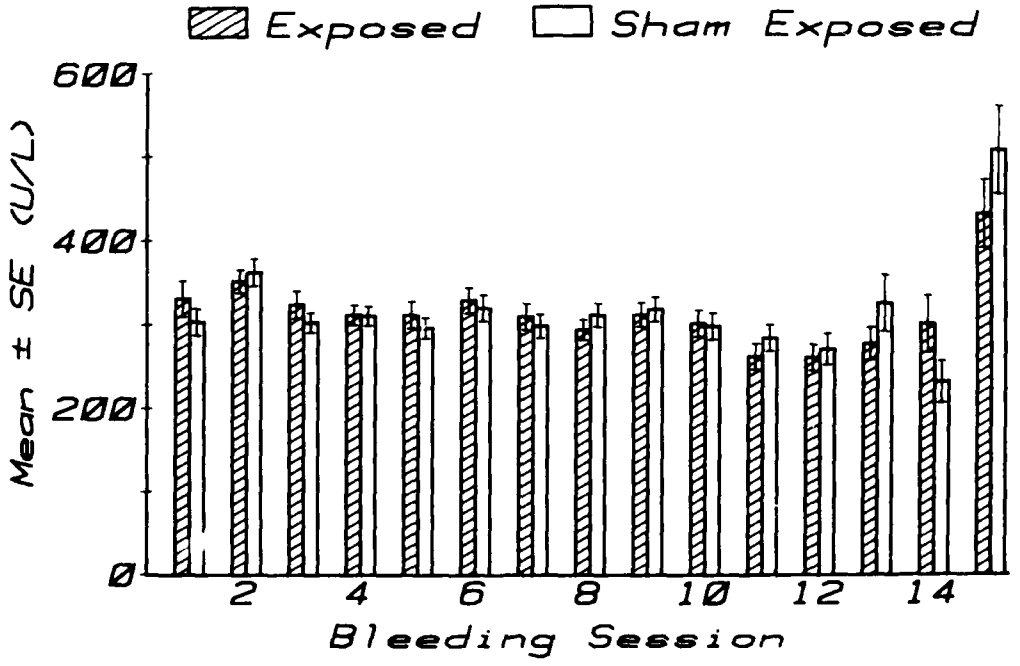

Fig. 26. Comparison of data on serum LDH for exposed and sham-exposed animals for 15 sampling sessions.

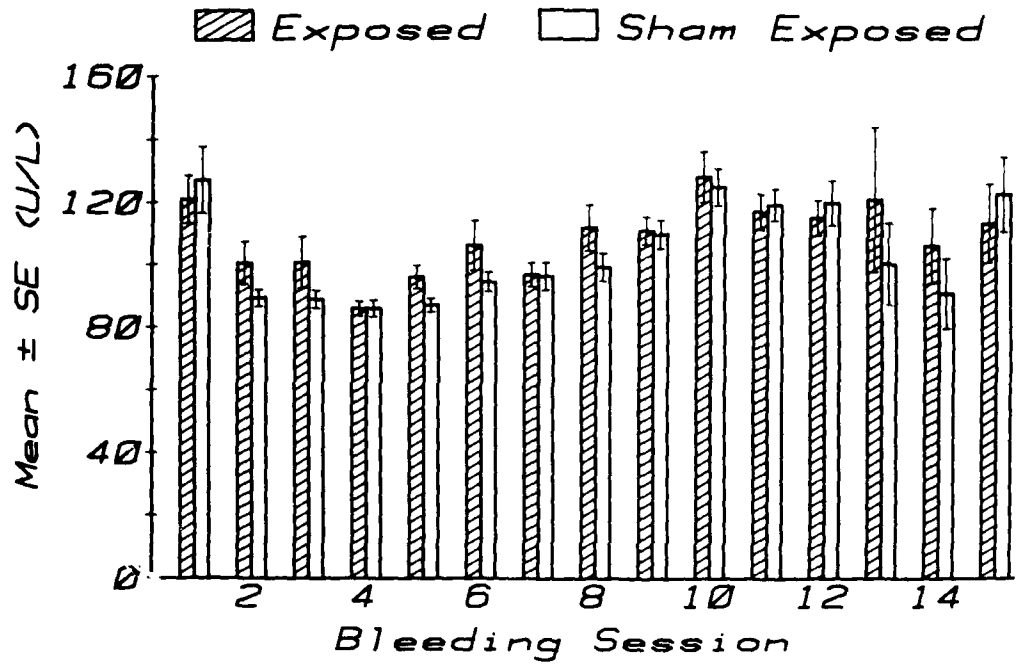

Fig. 27. Comparison of data on serum SGOT for exposed and sham-exposed animals for 15 sampling sessions.

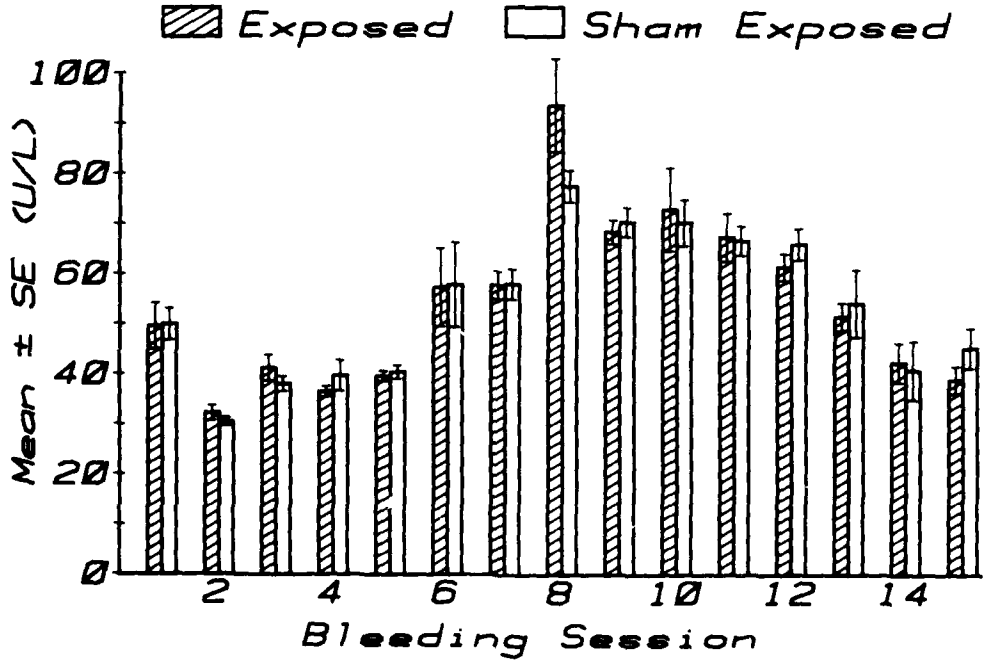

Fig. 28. Comparison of data on serum SGPT for exposed and sham-exposed animals for 15 sampling sessions.

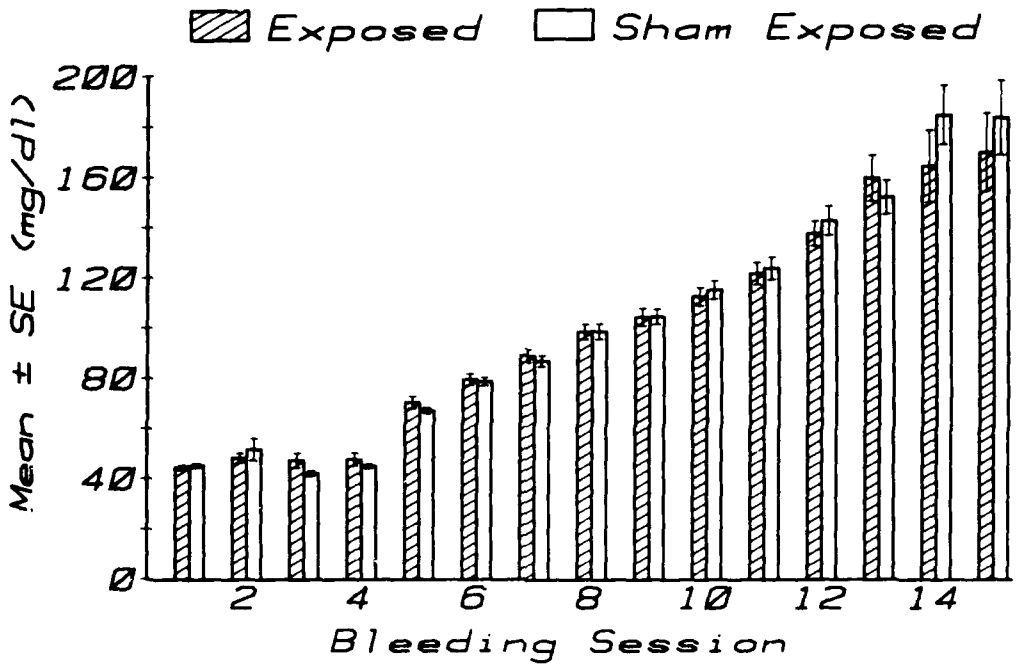

Fig. 29. Comparison of data on serum CHOLESTEROL for exposed and sham-exposed animals for 15 sampling sessions.

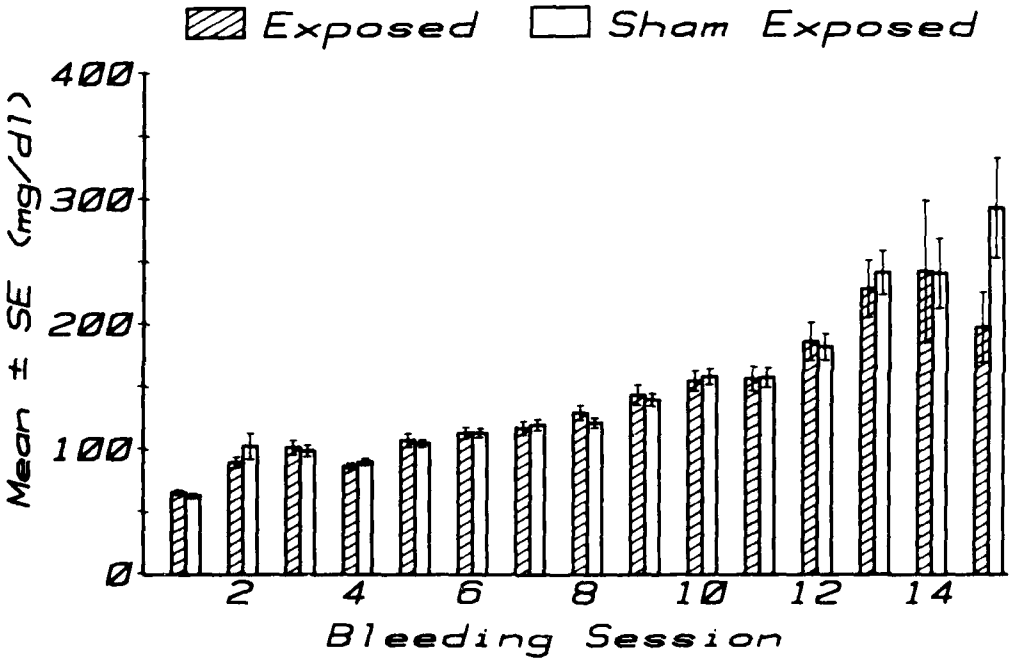

Fig. 30. Comparison of data on serum TRIGLYCERIDES for exposed and sham-exposed animals for 15 sampling sessions.

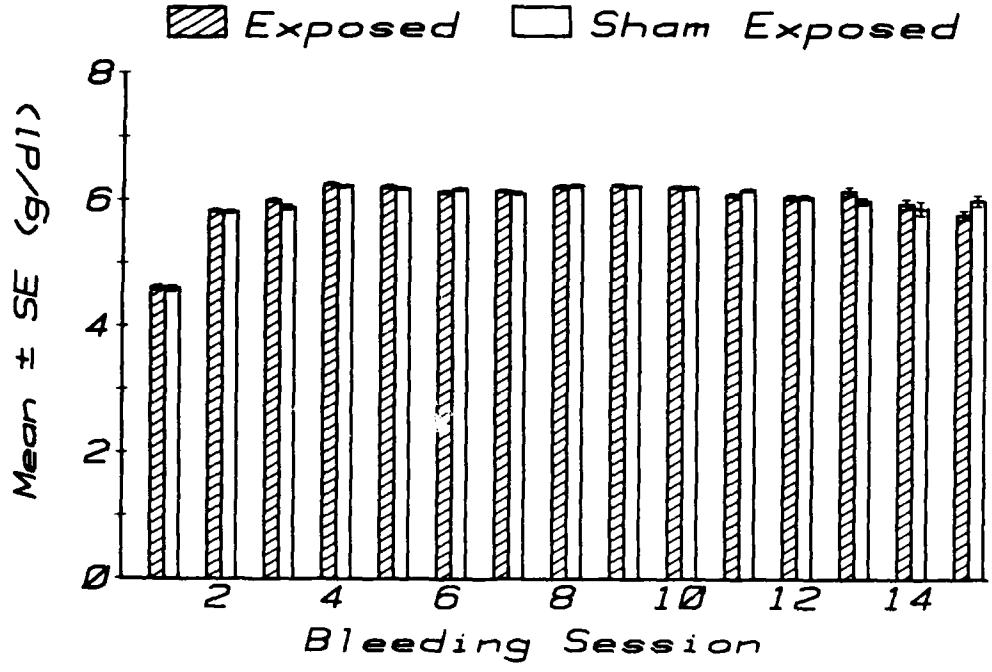

Fig. 31. Comparison of data on serum TOTAL PROTEIN for exposed and sham-exposed animals for 15 sampling sessions.

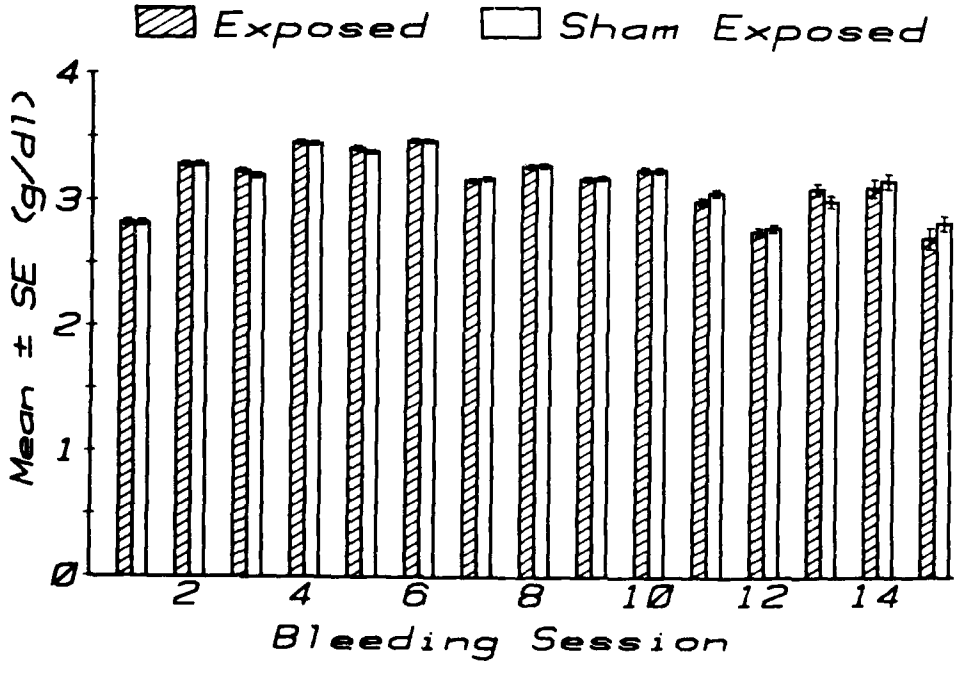

Fig. 32. Comparison of data on serum ALBUMIN for exposed and sham-exposed animals for 15 sampling sessions.

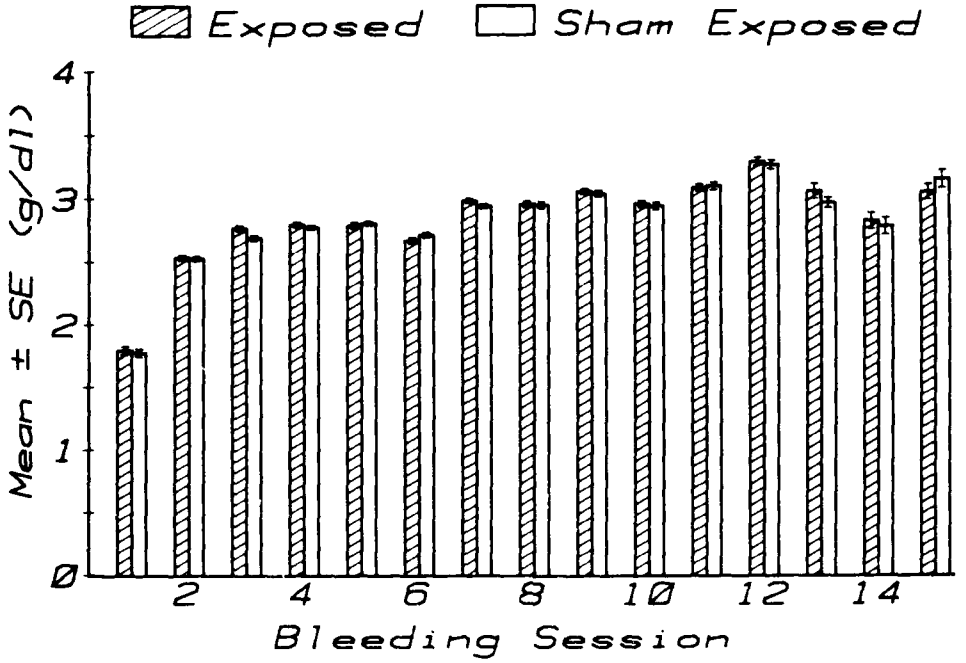

Fig. 33. Comparison of data on serum GLOBULIN for exposed and sham-exposed animals for 15 sampling sessions.

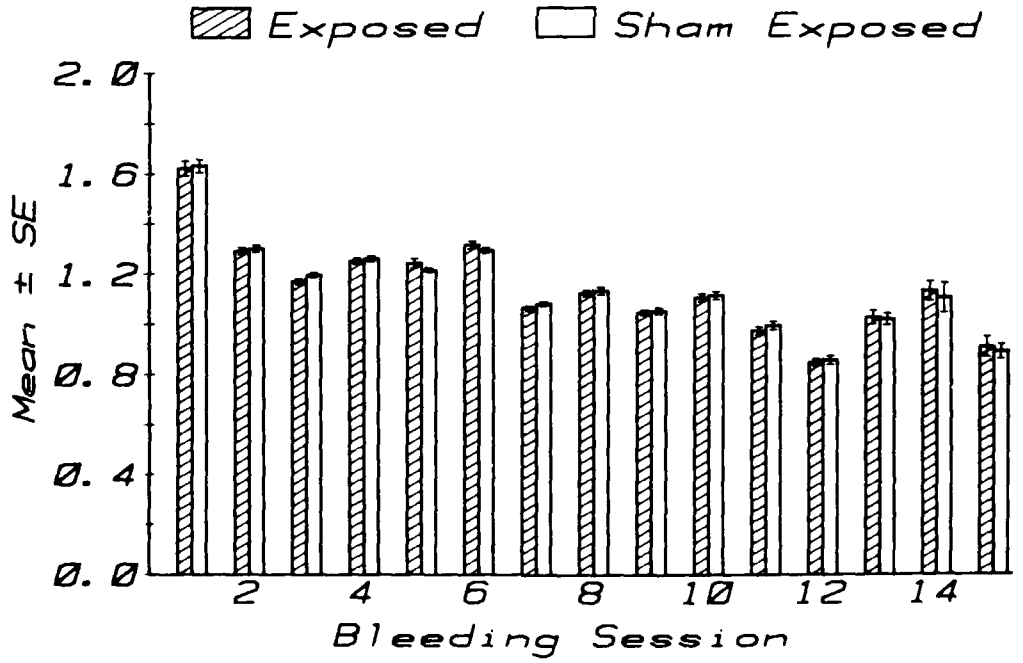

Fig. 34. Comparison of data on serum ALBUMIN/GLOBULIN RATIO for exposed and sham-exposed animals for 15 sampling sessions.

TABLE 12. SUMMARY OF USE OF DILUTION FACTOR BY SESSION AND TREATMENT CONDITION

EXPOSED

RUN	1:0	2:1	1:1	1:2	1:3	1:4	1:5	nbd[1]	mis[2]	qns[3]	dead
1	0	0	4	94	0	0	0	0	2	0	0
2	0	0	97	0	1	0	0	0	1	1	0
3	76	7	7	2	1	0	0	0	0	5	2
4	0	87	5	1	0	0	1	1	1	0	4
5	71	8	2	2	0	0	5	0	5	4	3
6	0	84	4	1	0	0	4	0	1	2	4
7	78	5	5	2	0	1	0	3	0	2	4
8	0	74	18	1	0	0	1	1	0	0	5
9	83	4	2	0	0	0	0	0	3	1	7
10	1	11	76	1	0	0	0	0	1	0	10
11	60	0	2	2	0	0	0	0	3	5	28
12	39	2	5	2	0	1	0	0	1	0	50
13	26	6	0	1	0	0	2	0	0	1	64
14	2	12	4	0	0	0	0	0	0	0	82
15	12	0	0	0	0	0	0	0	0	0	88

SHAM EXPOSED

RUN	1:0	2:1	1:1	1:2	1:3	1:4	1:5	nbd[1]	mis[2]	qns[3]	dead
1	0	0	3	97	0	0	0	0	0	0	0
2	0	0	99	0	0	0	0	0	1	0	0
3	89	3	5	0	1	0	0	0	1	0	1
4	0	91	1	1	0	0	2	2	0	0	3
5	77	8	0	2	0	0	0	0	5	4	4
6	0	83	2	0	0	0	1	1	1	5	7
7	76	4	5	3	0	0	0	1	2	1	3
8	1	78	7	1	0	1	0	0	1	0	11
9	77	7	2	0	0	0	0	0	3	1	10
10	0	12	67	3	0	0	1	0	3	0	14
11	54	1	6	2	0	0	1	0	4	3	29
12	40	6	2	2	0	2	0	0	1	0	47
13	27	5	2	2	0	0	2	0	0	1	61
14	3	14	1	0	0	0	0	0	0	0	82
15	13	0	0	0	0	0	0	0	0	0	87

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[1] No blood drawn.
[2] Missing data (failure of analyzer).
[3] Quantity not sufficient for determination.

TABLE 13. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM GLUCOSE FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	154.08	148.50	134.81	21.73	126.0	267.0	2.18
2	98	137.93	132.00	119.06	34.38	105.0	330.0	3.45
3	89	145.37	140.00	139.37	24.03	112.0	258.0	2.53
4	94	138.08	136.50	129.06	12.73	105.0	182.0	1.30
5	88	137.78	137.00	136.75	14.09	100.0	184.0	1.49
6	93	144.15	142.50	136.31	13.59	111.0	192.0	1.40
7	91	138.86	137.00	134.62	22.75	99.0	289.0	2.37
8	93	140.31	134.00	138.50	27.15	110.0	262.0	2.80
9	87	140.14	139.00	140.37	15.54	108.0	182.0	1.65
10	89	136.12	134.00	130.87	14.54	104.0	190.0	1.53
11	63	127.41	126.00	126.00	18.25	96.0	216.0	2.28
12	49	132.70	135.00	132.58	14.60	90.0	163.0	2.06
13	34	115.72	111.00	108.37	43.29	75.0	342.0	7.31
14	18	100.13	99.00	97.00	24.47	54.0	140.0	5.60
15	12	113.75	111.00	95.75	36.60	67.0	182.0	10.11

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	153.18	150.00	144.50	21.31	117.0	216.0	2.12
2	99	131.03	128.00	122.75	17.25	104.0	204.0	1.72
3	94	146.55	141.50	137.90	20.08	112.5	248.0	2.06
4	94	137.88	138.00	132.56	11.26	105.0	168.0	1.15
5	87	139.60	138.00	137.37	12.36	105.0	179.0	1.31
6	86	149.95	145.50	143.53	18.83	118.5	252.0	2.02
7	88	142.36	138.00	140.25	20.24	99.0	231.0	2.14
8	87	137.63	138.00	137.25	12.03	103.5	163.5	1.28
9	83	141.65	141.00	137.31	21.50	103.0	286.0	2.34
10	82	133.20	134.00	135.09	12.02	106.0	172.5	1.32
11	64	125.57	124.00	119.57	13.93	96.0	162.0	1.72
12	50	128.60	132.50	121.41	23.64	56.0	213.0	3.31
13	37	111.39	109.00	121.20	22.77	26.0	162.0	3.69
14	13	100.80	108.75	122.25	27.48	28.5	141.0	6.29
15	11	119.18	128.00	127.75	26.30	64.0	149.0	7.56

TABLE 14. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM BLOOD UREA NITROGEN FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	16.90	18.00	18.18	3.11	9.0	30.0	0.31
2	98	20.35	20.00	18.12	3.57	16.0	50.0	0.35
3	92	20.65	19.00	23.56	12.44	16.0	137.0	1.29
4	94	19.08	19.50	19.50	2.63	12.0	36.0	0.27
5	88	19.88	19.00	19.37	5.67	16.0	70.0	0.60
6	93	19.37	19.50	18.93	1.76	15.0	24.0	0.18
7	91	19.29	19.00	19.43	2.03	16.0	27.0	0.21
8	93	20.06	19.50	19.68	2.12	15.0	30.0	0.21
9	89	20.79	20.00	21.68	3.20	9.0	38.0	0.33
10	89	20.69	20.00	20.37	2.32	16.0	30.0	0.24
11	64	20.37	19.00	20.42	5.58	14.0	44.0	0.69
12	49	18.89	18.00	18.00	3.03	14.0	30.0	0.42
13	35	18.97	17.00	18.31	7.82	13.0	55.5	1.30
14	18	18.91	16.50	15.25	6.14	10.5	39.0	1.40
15	12	25.58	19.00	30.50	16.26	16.0	74.0	4.49

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	16.24	18.00	18.83	3.30	6.0	27.0	0.32
2	99	21.19	20.00	19.12	6.44	16.0	66.0	0.64
3	97	23.61	19.00	40.37	40.81	15.0	421.0	4.12
4	95	18.83	19.50	19.21	1.57	15.0	22.5	0.16
5	87	19.71	19.00	18.50	1.89	16.0	24.0	0.20
6	85	19.74	19.50	20.06	3.20	15.0	42.0	0.34
7	88	19.84	19.75	17.96	2.68	16.5	40.0	0.28
8	86	20.50	19.50	17.43	4.06	15.0	54.0	0.43
9	85	20.39	20.00	19.12	2.84	1.8	27.0	0.30
10	83	20.71	20.00	19.68	2.46	14.0	27.0	0.26
11	64	19.41	19.00	19.64	2.26	15.0	28.0	0.28
12	52	19.94	19.00	16.16	4.57	14.0	40.0	0.62
13	37	18.51	18.00	16.00	4.27	14.0	34.0	0.69
14	18	25.63	18.00	31.75	24.85	13.5	123.0	5.69
15	11	21.90	20.00	17.25	6.93	12.0	33.0	1.99

TABLE 15. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM CREATININE FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	96	0.71	0.90	0.82	0.31	0.0	1.2	0.03
2	98	0.52	0.60	0.56	0.18	0.0	1.0	0.01
3	93	0.58	0.50	0.51	0.35	0.3	3.8	0.03
4	94	0.49	0.45	0.37	0.15	0.0	1.2	0.01
5	88	0.61	0.50	0.45	0.37	0.3	2.8	0.04
6	93	0.57	0.60	0.52	0.23	0.0	1.2	0.02
7	91	0.55	0.50	0.51	0.10	0.4	1.0	0.01
8	93	0.61	0.60	0.58	0.12	0.4	1.0	0.01
9	89	0.60	0.60	0.61	0.08	0.4	0.9	0.00
10	89	0.55	0.60	0.55	0.13	0.0	0.8	0.01
11	64	0.67	0.70	0.55	0.12	0.4	1.1	0.01
12	42	0.59	0.50	0.27	0.39	0.0	2.7	0.06
13	35	0.60	0.60	0.55	0.23	0.4	1.6	0.03
14	18	0.71	0.60	0.72	0.18	0.6	1.3	0.04
15	12	0.84	0.70	0.92	0.45	0.5	2.2	0.12

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	0.85	0.60	1.35	2.14	0.0	21.6	0.21
2	99	0.56	0.60	0.52	0.18	0.0	1.2	0.01
3	97	0.58	0.50	0.68	0.45	0.4	4.9	0.04
4	95	0.48	0.45	0.45	0.13	0.0	0.7	0.01
5	87	0.56	0.50	0.53	0.30	0.4	2.5	0.03
6	83	0.56	0.60	0.59	0.23	0.0	1.3	0.02
7	88	0.55	0.50	0.51	0.10	0.4	1.0	0.01
8	87	0.63	0.60	0.64	0.16	0.4	1.5	0.01
9	85	0.59	0.60	0.57	0.08	0.5	0.9	0.01
10	82	0.55	0.60	0.61	0.12	0.2	0.8	0.01
11	64	0.68	0.60	0.60	0.16	0.4	1.2	0.02
12	47	0.52	0.50	0.40	0.28	0.0	1.3	0.04
13	37	0.62	0.50	0.58	0.34	0.4	2.2	0.05
14	18	0.89	0.67	1.10	0.88	0.4	4.3	0.20
15	11	0.71	0.60	0.62	0.17	0.5	1.0	0.05

TABLE 16. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM SODIUM FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	35	145.20	147.00	146.25	4.25	135.0	153.0	0.72
2	36	144.66	144.00	143.80	1.78	142.0	148.0	0.29
3	92	141.59	142.50	144.25	4.72	125.0	153.0	0.49
4	93	144.93	144.00	144.65	4.93	136.5	180.0	0.50
5	88	143.79	143.00	143.62	4.88	138.0	168.0	0.51
6	92	143.88	142.50	145.31	5.22	123.0	174.0	0.54
7	90	144.83	145.00	145.43	2.13	138.0	155.0	0.22
8	89	143.11	142.50	143.75	2.86	128.0	156.0	0.30
9	89	145.38	145.00	145.87	1.54	139.0	149.0	0.16
10	32	142.23	142.00	139.50	3.24	138.0	150.0	0.56
11	63	145.41	146.00	147.00	2.51	138.0	150.0	0.31
12	47	146.55	146.00	145.90	2.43	142.0	155.0	0.35
13	34	146.41	145.50	146.12	4.42	143.0	168.0	0.74
14	18	146.80	147.50	148.50	3.81	133.5	151.5	0.87
15	12	143.41	143.00	142.50	2.35	140.0	150.0	0.65

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	35	145.45	147.00	147.75	3.59	132.0	150.0	0.59
2	38	144.42	144.00	145.00	2.13	138.0	148.0	0.34
3	93	141.65	143.00	142.06	4.09	128.0	153.0	0.42
4	95	144.76	144.00	142.31	4.53	135.0	174.0	0.46
5	87	142.59	143.00	142.68	2.17	137.0	150.0	0.23
6	86	143.59	142.50	141.37	3.35	138.0	156.0	0.36
7	88	144.40	145.00	146.34	4.42	106.5	149.0	0.46
8	86	143.47	144.00	143.25	1.99	138.0	150.0	0.21
9	83	145.34	146.00	146.06	1.75	141.0	150.0	0.19
10	32	142.89	144.00	146.50	3.42	136.0	148.0	0.59
11	61	145.95	146.00	142.00	5.90	138.0	186.0	0.75
12	51	146.05	146.00	148.25	3.93	141.0	170.0	0.54
13	37	146.04	145.50	140.00	8.03	111.0	169.0	1.30
14	17	146.82	147.00	148.25	4.36	132.0	151.5	1.02
15	11	142.54	143.00	142.75	1.57	139.0	144.0	0.45

TABLE 17. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM POTASSIUM FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	65	5.94	5.70	5.65	0.92	4.5	9.9	0.11
2	98	5.21	5.20	4.83	0.64	3.8	9.3	0.06
3	93	5.36	5.30	5.33	0.71	4.4	9.4	0.07
4	94	5.25	5.25	5.45	0.52	4.2	8.2	0.05
5	88	5.30	5.2[illegible]	5.23	0.53	4.5	8.4	0.05
6	92	4.88	4.80	4.90	0.34	4.0	6.0	0.03
7	91	4.91	4.90	4.91	0.32	4.2	6.5	0.03
8	93	4.68	4.65	4.32	0.45	3.8	6.6	0.04
9	89	4.91	4.90	4.69	0.36	4.3	6.4	0.03
10	89	4.49	4.40	4.67	0.43	3.8	5.8	0.04
11	64	4.64	4.60	4.45	0.43	3.6	6.0	0.05
12	44	4.69	4.60	4.41	0.54	3.6	6.3	0.08
13	35	4.45	4.30	4.72	0.72	3.3	7.1	0.12
14	13	4.20	4.20	4.35	0.41	3.6	5.1	0.09
15	12	5.83	5.80	5.52	0.67	4.9	7.4	0.18

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	[illegible]	5.[illegible]	5.[illegible]	4.93	0.66	4.2	7.5	0.0[illegible]
2	99	5.16	5.00	5.00	0.37	4.6	6.4	0.04
3	97	5.28	5.20	5.03	0.41	3.9	6.5	0.04
4	95	5.23	5.25	5.15	0.38	4.5	6.6	0.04
5	87	5.20	5.20	5.24	0.30	4.4	5.9	0.03
6	86	4.95	4.95	5.09	0.40	4.2	7.0	0.04
7	87	4.89	4.90	4.90	0.34	4.0	6.0	0.03
8	86	4.76	4.72	4.70	0.32	4.0	5.5	0.03
9	84	4.90	4.90	4.59	0.37	4.0	5.9	0.04
10	92	4.43	4.40	4.05	0.43	3.6	6.0	0.04
11	64	4.73	4.70	4.67	0.52	3.6	6.6	0.06
12	50	4.59	4.50	4.32	0.55	3.5	6.8	0.07
13	36	4.51	4.35	4.13	0.75	3.7	8.0	0.12
14	18	4.05	3.97	3.65	0.54	3.3	5.4	0.12
15	11	6.21	6.10	5.60	0.64	5.1	7.1	0.18

TABLE 18. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM CHLORIDE FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	49.85	51.00	43.50	8.72	12.0	84.0	0.87
2	98	77.99	78.00	77.18	7.37	39.0	86.0	0.74
3	91	95.75	99.00	102.93	8.60	57.0	106.0	0.39
4	92	89.00	91.50	87.75	9.60	39.0	99.0	0.99
5	85	95.08	100.00	96.56	18.56	0.0	103.0	2.00
6	92	95.53	99.00	101.34	13.83	24.0	106.5	1.43
7	91	100.14	101.00	102.18	5.20	60.0	105.0	0.54
8	92	96.07	97.50	94.03	7.44	40.0	106.5	0.77
9	86	101.14	101.50	102.56	1.95	92.0	105.0	0.21
10	89	90.73	90.00	86.81	5.12	75.0	102.0	0.54
11	64	99.54	101.00	103.14	6.14	66.0	106.0	0.76
12	49	97.55	101.00	101.16	14.43	15.0	109.0	2.04
13	34	96.80	99.00	96.37	11.35	36.0	105.0	1.91
14	18	99.02	100.50	102.50	3.92	90.0	105.0	0.89
15	12	100.33	99.50	98.75	2.34	97.0	104.0	0.64

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	49.41	48.00	50.72	8.36	27.0	88.0	0.83
2	99	78.05	80.00	79.37	7.70	42.0	88.0	0.77
3	94	96.76	99.00	100.50	8.54	48.0	104.0	0.87
4	93	90.67	91.50	89.90	4.79	57.0	97.5	0.49
5	87	98.80	100.00	97.68	5.46	66.0	105.0	0.58
6	86	98.34	99.00	97.03	8.23	30.0	112.5	0.88
7	87	100.06	101.00	102.84	4.83	70.5	105.0	0.51
8	87	96.30	97.50	98.43	11.73	0.0	105.0	1.25
9	83	100.75	101.00	101.06	2.66	84.0	105.0	0.29
10	82	88.62	90.00	95.62	11.70	0.0	102.0	1.28
11	64	98.03	101.00	99.21	10.86	24.0	105.0	1.34
12	52	94.89	101.00	96.66	19.13	5.0	105.0	2.62
13	34	96.70	98.50	95.50	9.24	63.0	115.0	1.56
14	18	99.83	100.50	103.00	4.52	85.5	106.5	1.03
15	11	99.36	99.00	97.50	1.85	96.0	102.0	0.53

TABLE 19. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM CARBON DIOXIDE FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	13.77	15.00	15.37	3.72	3.0	21.0	0.37
2	96	24.91	26.00	25.31	2.59	15.0	30.0	0.26
3	88	26.69	27.00	27.62	2.49	18.0	32.0	0.26
4	94	25.33	25.50	28.12	4.57	0.0	30.0	0.46
5	88	25.91	27.00	25.18	5.40	0.0	31.0	0.57
6	90	23.37	24.00	24.37	4.99	0.0	30.0	0.52
7	91	25.47	26.00	28.12	4.07	0.0	30.0	0.42
8	93	23.59	24.00	23.15	3.86	0.0	28.5	0.39
9	89	25.82	26.00	25.62	1.82	20.0	30.0	0.19
10	87	21.51	22.00	19.00	2.95	14.0	30.0	0.31
11	63	26.81	27.00	27.75	2.63	18.0	31.0	0.32
12	49	27.35	28.00	29.75	4.28	5.0	32.0	0.60
13	35	26.88	29.00	28.87	8.01	0.0	33.0	1.33
14	18	27.00	27.00	27.00	2.72	22.0	32.0	0.62
15	12	26.16	26.50	27.25	2.03	22.0	29.0	0.56

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	13.81	15.00	15.72	3.91	2.0	21.0	0.39
2	98	24.94	26.00	23.43	2.43	15.0	30.0	0.24
3	92	26.85	27.00	25.81	2.66	10.0	33.0	0.27
4	95	25.95	27.00	28.12	4.30	0.0	30.0	0.43
5	87	27.24	27.00	28.56	2.09	18.0	31.0	0.22
6	84	23.94	24.00	22.68	3.42	0.0	33.0	0.37
7	98	25.50	26.00	26.62	3.07	12.0	30.0	0.32
8	87	24.01	24.00	25.03	2.47	10.0	28.5	0.26
9	85	25.60	26.00	26.62	2.72	12.0	30.0	0.29
10	82	20.67	21.00	21.12	3.36	0.0	26.0	0.37
11	63	26.07	27.00	28.91	3.62	6.0	31.0	0.45
12	52	26.78	28.00	29.29	5.29	5.0	31.5	0.72
13	36	27.26	30.00	29.70	7.51	0.0	33.0	1.23
14	18	27.72	28.50	29.91	2.79	22.0	31.5	0.64
15	11	24.81	25.00	26.25	2.35	21.0	28.0	0.67

TABLE 20. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM URIC ACID FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	1.59	1.50	1.38	0.65	0.6	4.8	0.06
2	98	1.15	1.00	0.92	0.74	0.6	5.8	0.07
3	93	1.02	1.00	0.95	0.46	0.7	4.8	0.04
4	94	0.94	0.90	0.84	0.24	0.0	1.5	0.02
5	83	0.95	0.90	0.82	0.17	0.6	1.8	0.01
6	83	0.92	0.90	0.67	0.23	0.6	1.8	0.02
7	90	1.03	0.90	0.94	0.58	0.6	6.1	0.06
8	93	1.02	0.90	0.84	0.48	0.6	4.5	0.05
9	88	0.95	0.90	0.94	0.22	0.6	1.7	0.02
10	89	0.86	1.00	0.87	0.39	0.0	2.0	0.04
11	64	0.91	0.85	0.74	0.29	0.6	2.6	0.03
12	49	0.97	0.90	0.97	0.19	0.7	1.3	0.02
13	35	1.09	0.90	1.40	1.04	0.6	7.0	0.17
14	18	1.03	0.95	0.82	0.30	0.6	1.9	0.07
15	12	1.70	1.65	1.52	0.63	0.9	3.4	0.17

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	1.60	1.50	1.20	0.59	0.6	4.2	0.06
2	99	1.00	1.00	1.08	0.31	0.6	3.2	0.03
3	97	1.00	1.00	0.83	0.27	0.7	2.9	0.02
4	95	0.96	0.90	0.92	0.20	0.6	1.6	0.02
5	84	0.96	0.90	0.92	0.19	0.6	1.6	0.02
6	74	1.05	0.90	0.99	0.63	0.6	6.1	0.07
7	86	1.02	0.90	0.78	0.46	0.6	3.5	0.04
8	87	1.06	1.05	0.91	0.54	0.6	5.6	0.05
9	81	0.92	0.90	0.82	0.40	0.6	4.2	0.04
10	83	0.88	0.90	1.00	0.47	0.0	3.2	0.05
11	64	0.85	0.90	0.90	0.15	0.6	1.2	0.01
12	52	1.04	0.90	0.85	0.48	0.6	3.7	0.06
13	37	1.20	0.90	1.45	1.40	0.6	9.1	0.22
14	18	0.93	0.90	1.10	0.17	0.6	1.2	0.04
15	11	1.50	1.40	1.22	0.29	1.0	1.9	0.08

TABLE 21. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM CALCIUM FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	10.07	10.20	9.88	0.49	8.7	11.4	0.05
2	98	9.55	9.60	9.29	0.32	8.7	10.6	0.03
3	92	9.81	9.80	9.63	0.69	8.1	13.0	0.07
4	93	9.83	9.90	9.86	0.42	7.8	10.8	0.04
5	87	9.87	9.80	9.73	0.23	9.0	10.3	0.02
6	92	9.95	10.05	10.00	0.37	8.2	10.8	0.03
7	91	10.10	10.10	9.89	0.33	9.3	11.2	0.03
8	93	10.17	10.20	10.32	0.39	7.6	10.9	0.04
9	89	9.98	10.00	9.83	0.31	9.2	10.6	0.03
10	88	10.07	10.00	10.23	0.31	9.0	10.8	0.03
11	64	10.21	10.20	10.28	0.37	9.0	11.0	0.04
12	49	10.27	10.30	10.50	0.35	9.0	11.0	0.05
13	35	10.21	10.10	10.42	0.54	9.0	12.8	0.09
14	18	10.10	10.12	9.90	0.48	8.8	10.9	0.11
15	12	10.06	9.95	9.72	0.47	9.3	11.0	0.13

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	10.04	9.90	10.10	0.58	7.6	11.7	0.05
2	99	9.54	9.60	9.68	0.30	8.1	10.4	0.03
3	96	9.79	9.60	9.80	0.96	8.6	15.0	0.09
4	95	9.85	9.90	9.65	0.39	7.8	10.5	0.04
5	87	9.87	9.90	9.82	0.26	9.3	10.5	0.02
6	86	10.03	10.05	9.97	0.24	9.4	10.6	0.02
7	88	10.08	10.10	10.05	0.34	8.4	10.8	0.03
8	87	10.15	10.05	10.02	0.27	9.6	10.9	0.02
9	85	10.02	10.10	10.03	0.26	9.3	10.6	0.02
10	83	10.02	10.00	10.01	0.27	9.4	10.8	0.03
11	63	10.26	10.30	10.35	0.35	9.0	10.8	0.04
12	52	10.20	10.30	10.42	0.35	9.0	10.9	0.04
13	37	10.22	10.20	9.58	0.84	9.0	14.8	0.13
14	18	10.39	10.35	10.37	0.68	9.1	12.6	0.15
15	11	10.17	10.20	9.95	0.28	9.7	10.7	0.08

TABLE 22. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM PHOSPHORUS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	9.78	9.90	9.52	1.42	4.8	13.2	0.14
2	97	7.52	7.60	7.37	0.62	5.8	9.4	0.06
3	86	6.12	6.10	5.96	0.85	4.8	11.0	0.09
4	93	5.96	6.00	5.91	0.55	4.8	7.3	0.05
5	86	5.60	5.57	5.36	0.55	4.4	7.5	0.06
6	86	5.33	5.40	5.71	0.41	3.7	6.6	0.04
7	88	5.18	5.20	5.21	0.42	4.3	6.4	0.04
8	93	5.25	5.25	5.11	0.47	3.6	7.0	0.04
9	89	4.78	4.80	4.73	0.38	3.5	5.7	0.04
10	89	5.07	5.10	5.25	0.39	4.2	6.6	0.04
11	62	4.69	4.70	4.72	0.41	3.5	5.6	0.05
12	44	4.71	4.70	4.70	0.34	4.0	5.4	0.05
13	35	4.93	4.60	5.13	1.49	4.0	13.1	0.24
14	18	4.71	4.50	4.50	0.49	4.2	6.0	0.11
15	12	5.05	4.95	4.65	1.03	3.6	7.8	0.28

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	99	9.85	9.90	10.35	1.34	5.4	12.6	0.13
2	99	7.44	7.40	7.46	0.51	6.4	9.8	0.05
3	93	6.17	6.00	6.14	2.01	4.9	24.8	0.20
4	95	5.94	6.00	5.83	0.52	4.6	7.3	0.05
5	87	5.40	5.40	5.47	0.41	4.6	6.6	0.04
6	81	5.29	5.25	5.33	0.40	4.3	6.6	0.04
7	84	5.06	5.10	5.40	0.41	4.1	6.0	0.04
8	85	5.11	5.10	4.81	0.45	3.3	6.0	0.04
9	84	4.87	4.90	4.78	0.41	3.4	6.5	0.04
10	82	5.02	5.00	4.93	0.43	3.8	6.4	0.04
11	62	4.75	4.75	4.90	0.42	3.7	6.6	0.05
12	47	4.78	4.80	4.54	0.49	3.7	6.5	0.07
13	35	5.38	4.60	5.74	2.33	4.1	17.2	0.47
14	18	5.01	4.65	5.00	1.41	3.9	10.5	0.32
15	11	4.63	4.60	4.50	0.57	4.0	6.0	0.16

TABLE 23. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM ALKALINE PHOSPHATASE FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	586.22	603.00	604.12	157.74	135.0	969.0	15.85
2	98	228.34	230.00	231.12	31.78	140.0	302.0	3.19
3	92	205.50	204.75	214.25	34.67	104.0	300.0	3.59
4	93	215.47	214.00	201.93	32.64	99.0	282.0	3.36
5	88	197.17	197.00	220.75	37.63	86.0	282.0	3.98
6	93	212.40	210.00	216.93	42.89	133.5	400.5	4.42
7	91	196.90	194.00	185.68	37.79	58.0	285.0	3.94
8	92	195.80	189.75	177.84	43.27	109.5	474.0	4.48
9	89	186.29	188.00	197.12	28.00	115.0	261.0	2.95
10	88	194.32	191.00	192.12	37.42	114.0	364.0	3.96
11	64	174.35	174.00	163.50	30.55	88.0	239.0	3.79
12	49	196.68	200.00	200.00	32.35	120.0	265.0	4.57
13	35	192.02	184.00	230.50	74.40	34.0	558.0	12.39
14	18	153.50	146.25	153.00	37.97	63.0	243.0	8.69
15	12	112.50	108.50	88.50	21.19	71.0	141.0	5.85

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	595.75	619.50	586.00	143.99	135.0	873.0	14.32
2	99	231.73	230.00	218.00	28.94	148.0	308.0	2.89
3	97	202.20	204.00	224.50	34.06	109.0	277.0	3.44
4	94	214.67	212.25	210.84	34.37	88.5	306.0	3.52
5	87	194.20	193.00	195.28	28.75	91.5	276.0	3.06
6	86	205.76	207.00	193.40	31.86	93.0	271.5	3.41
7	88	197.56	196.75	202.12	34.80	22.0	284.0	3.68
8	87	196.48	190.00	205.78	65.61	84.0	733.5	6.99
9	84	184.98	181.50	161.34	32.58	129.0	301.5	3.53
10	83	189.66	186.00	181.62	27.83	102.0	284.0	3.03
11	64	186.52	184.50	184.92	33.25	131.0	282.0	4.12
12	52	194.49	191.00	179.50	30.18	117.0	267.0	4.14
13	37	172.71	174.00	157.50	33.00	67.0	248.0	5.35
14	18	152.19	154.50	151.50	27.85	102.0	201.0	6.38
15	11	119.90	125.00	134.75	23.79	77.0	154.0	6.84

TABLE 24. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM LDH FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	331.70	285.00	323.81	213.90	60.0	1467.0	21.60
2	96	352.58	350.00	316.93	138.42	136.0	1101.0	14.05
3	90	323.74	292.50	257.93	157.87	53.0	1146.0	16.54
4	93	311.15	296.00	299.18	117.67	87.0	766.0	12.13
5	82	310.82	298.00	265.68	146.59	93.0	1014.0	16.09
6	89	328.48	302.00	263.25	146.13	108.0	936.0	15.40
7	90	308.94	270.00	246.25	153.43	94.0	906.0	16.08
8	92	293.71	267.00	211.03	120.99	100.5	690.0	12.54
9	88	311.90	285.50	213.62	137.43	94.0	732.0	14.56
10	89	300.80	272.00	207.75	151.82	72.0	796.0	16.00
11	64	261.09	223.00	231.42	126.03	84.0	772.0	15.63
12	48	259.59	233.50	150.50	111.27	113.0	488.0	15.89
13	35	276.02	246.00	299.87	118.29	109.0	618.0	19.70
14	18	300.16	271.50	221.91	148.92	127.5	694.0	34.11
15	11	431.90	443.00	374.00	141.35	270.0	686.0	40.63

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	96	303.87	294.00	251.06	159.73	63.0	1066.0	16.21
2	96	362.91	343.00	299.50	163.69	124.0	1060.0	16.62
3	96	302.30	294.00	149.81	118.82	119.0	612.0	12.06
4	95	309.71	300.00	294.84	115.30	96.0	550.5	11.76
5	83	295.48	282.00	300.81	114.89	100.0	559.0	12.53
6	84	319.26	319.50	371.53	145.97	90.0	733.5	15.83
7	86	298.03	289.00	310.62	133.26	80.0	818.0	14.28
8	84	311.26	318.75	355.87	131.02	75.0	717.0	14.21
9	84	318.08	299.50	340.18	138.00	118.0	829.0	14.96
10	83	297.76	258.00	228.00	148.80	84.0	852.0	16.23
11	64	283.15	263.00	140.00	128.82	97.0	699.0	15.97
12	52	259.72	236.00	145.66	137.79	78.0	890.0	18.92
13	37	324.23	282.00	252.30	209.53	147.0	1200.0	33.97
14	18	230.75	217.50	165.75	109.82	100.5	492.0	25.15
15	11	507.63	522.00	394.50	182.03	233.0	879.0	52.33

TABLE 25. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM SGOT FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	121.05	105.00	105.00	76.40	54.0	582.0	7.72
2	97	100.49	84.00	79.12	68.31	50.0	516.0	6.90
3	92	100.64	82.50	97.12	80.14	53.0	759.0	8.31
4	93	85.82	82.50	92.34	23.20	30.0	229.5	2.39
5	87	96.05	88.00	94.87	35.52	42.0	324.0	3.78
6	92	106.20	88.50	89.90	77.35	48.0	718.5	8.02
7	82	96.82	88.50	77.93	34.80	47.0	212.0	3.82
8	91	111.78	94.50	73.12	70.51	40.5	562.5	7.35
9	89	110.84	104.00	91.87	42.22	39.0	321.0	4.45
10	89	127.86	110.00	83.12	76.85	44.0	670.0	8.10
11	63	116.85	110.00	94.50	45.15	41.0	255.0	5.64
12	49	115.04	102.00	83.75	40.05	40.0	215.0	5.66
13	35	120.85	85.00	153.12	138.13	49.0	882.0	23.01
14	18	106.13	87.00	69.58	52.36	37.5	230.0	11.99
15	12	113.33	102.50	102.00	45.53	65.0	213.0	12.58

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	99	127.16	108.00	99.75	106.77	51.0	831.0	10.67
2	98	89.34	81.00	75.12	27.19	44.0	210.0	2.73
3	97	88.75	83.00	88.18	28.90	58.0	219.0	2.92
4	95	85.99	79.50	67.31	26.79	57.0	222.0	2.73
5	87	87.14	82.00	83.25	20.82	57.0	197.0	2.21
6	85	94.52	91.50	108.00	29.22	49.5	268.5	3.15
7	84	96.26	89.50	89.87	40.72	46.0	280.0	4.41
8	84	99.19	90.00	100.46	41.48	20.0	277.5	4.50
9	83	109.65	98.00	83.62	42.28	51.0	225.0	4.61
10	82	124.63	113.00	78.93	53.98	64.0	303.0	5.92
11	64	119.08	108.00	94.92	41.08	57.0	234.0	5.09
12	52	119.84	105.00	76.50	51.91	57.0	291.0	7.12
13	37	100.36	76.00	79.95	80.96	39.0	448.5	13.12
14	18	90.88	77.25	76.50	49.36	45.0	234.0	11.30
15	11	122.54	120.00	102.50	41.45	66.0	212.0	11.91

TABLE 26. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM SGPT FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	49.60	42.00	42.93	46.33	15.0	462.0	4.65
2	95	32.31	30.00	34.62	15.07	14.0	124.0	1.53
3	90	41.21	36.00	37.71	25.00	25.5	221.0	2.62
4	89	36.48	37.50	34.40	10.44	1.5	60.0	1.10
5	88	39.53	38.00	32.37	9.88	20.0	86.0	1.04
6	93	57.32	46.50	70.40	76.35	24.0	766.5	7.87
7	89	57.71	51.00	41.18	26.67	29.0	224.0	2.81
8	89	93.56	76.50	83.06	87.55	39.0	744.0	9.22
9	89	68.47	63.00	58.68	22.69	39.0	144.0	2.39
10	81	72.90	60.00	68.37	74.77	28.0	674.0	8.25
11	64	67.37	56.50	53.92	38.67	33.0	326.0	4.79
12	49	61.38	56.00	55.00	19.16	34.0	118.0	2.71
13	35	51.45	46.50	38.25	16.70	30.0	96.0	2.78
14	18	42.33	35.25	33.08	17.29	22.0	88.5	3.96
15	12	38.83	37.00	31.00	9.87	22.0	58.0	2.72

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	50.01	45.00	31.87	32.04	18.0	240.0	3.22
2	98	30.53	30.00	30.37	8.66	18.0	84.0	0.87
3	97	38.03	35.00	35.00	14.93	22.0	132.0	1.51
4	90	39.63	36.00	18.00	29.67	0.0	288.0	3.11
5	87	40.33	39.00	37.87	13.06	15.0	137.0	1.39
6	85	57.81	46.50	68.43	78.74	22.5	757.5	8.49
7	87	57.96	50.00	43.56	28.61	33.0	202.0	3.05
8	85	77.56	69.00	75.59	29.56	38.0	238.5	3.13
9	84	70.37	63.00	48.12	26.95	39.0	185.0	2.92
10	80	70.28	56.00	43.42	41.42	28.0	244.0	4.60
11	64	66.66	62.50	57.50	23.80	32.0	151.0	2.95
12	52	65.97	58.75	55.00	23.23	29.0	133.0	3.19
13	37	54.10	45.00	49.95	41.67	24.0	283.5	6.75
14	18	40.75	33.75	45.75	25.63	27.0	139.5	5.97
15	11	45.18	46.00	36.25	14.08	25.0	70.0	4.04

TABLE 27. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM CHOLESTEROL FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	44.63	45.00	51.18	10.55	12.0	69.0	1.06
2	98	48.56	44.00	41.62	19.80	30.0	216.0	1.99
3	93	47.60	43.00	39.81	28.77	25.0	262.0	2.96
4	94	47.95	44.25	43.68	25.42	28.5	271.5	2.60
5	88	70.66	66.00	57.12	23.05	46.0	224.0	2.44
6	93	79.80	72.00	71.90	22.10	48.0	175.5	2.28
7	91	89.07	82.00	82.31	25.90	57.0	192.0	2.70
8	93	98.58	93.00	78.09	29.95	48.0	208.5	3.09
9	89	104.38	96.00	89.31	33.92	58.0	225.0	3.57
10	89	112.55	104.00	92.37	35.02	62.0	224.0	3.69
11	64	121.56	111.00	98.07	36.12	67.0	212.0	4.48
12	49	137.59	126.00	115.75	35.31	80.0	223.0	4.99
13	32	159.46	143.00	113.87	52.50	89.0	288.0	9.13
14	18	164.02	150.75	99.75	62.63	57.0	313.5	14.34
15	12	169.58	156.50	139.25	56.03	99.0	260.0	15.48

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	45.52	45.00	45.50	9.38	21.0	84.0	0.93
2	98	51.98	44.50	54.00	44.57	32.0	384.0	4.48
3	98	42.34	41.00	44.68	11.51	25.0	130.0	1.15
4	95	45.31	45.00	39.00	9.15	30.0	78.0	0.93
5	87	67.47	66.00	61.81	11.43	44.0	101.0	1.21
6	86	78.85	75.00	70.87	16.22	54.0	144.0	1.74
7	98	86.97	84.00	73.06	21.09	53.0	160.0	2.23
8	87	98.78	93.00	90.93	29.67	57.0	238.0	3.16
9	85	104.50	98.00	82.43	28.87	56.0	197.0	3.11
10	83	115.20	106.00	94.00	33.61	60.0	220.0	3.66
11	64	123.74	115.50	105.23	36.95	62.0	264.0	4.58
12	52	142.65	134.50	111.25	42.68	63.0	256.0	5.86
13	30	151.85	145.50	143.87	37.68	85.0	242.0	6.76
14	18	184.36	162.75	149.00	50.34	117.0	309.0	11.64
15	11	183.18	167.00	130.75	51.11	90.0	252.0	14.69

TABLE 28. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM TRIGLYCERIDES FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	92	65.88	63.00	58.12	18.79	30.0	120.0	1.94
2	98	89.92	82.00	97.50	45.07	30.0	390.0	4.53
3	92	102.04	94.00	77.53	57.02	49.5	498.0	5.91
4	93	87.17	88.50	98.15	23.16	34.5	180.0	2.38
5	87	107.52	96.00	96.25	51.50	40.0	340.0	5.49
6	92	113.57	101.25	101.81	45.31	45.0	348.0	4.69
7	87	117.65	104.00	103.71	52.42	38.0	388.5	5.58
8	87	129.41	120.00	120.28	56.75	48.0	433.5	6.05
9	84	143.73	124.50	133.00	75.63	37.0	549.0	8.20
10	88	155.27	135.00	89.25	77.49	66.0	438.0	8.21
11	64	156.95	131.50	132.85	78.84	51.0	433.0	9.77
12	48	186.20	156.50	110.60	107.48	50.0	656.0	15.35
13	35	228.28	204.00	151.68	137.51	67.5	741.0	22.90
14	18	242.19	146.75	230.50	247.76	60.0	1083.0	56.75
15	12	197.41	192.50	172.00	101.74	84.0	436.0	28.12

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	62.99	60.00	57.75	19.70	21.0	105.0	1.99
2	98	103.03	88.00	90.62	104.98	36.0	910.0	10.55
3	96	99.13	93.50	118.81	47.29	35.0	482.0	4.80
4	95	90.42	90.00	103.50	27.76	36.0	156.0	2.83
5	93	105.47	105.00	102.75	27.41	38.0	186.0	2.99
6	85	113.30	108.00	107.71	34.90	51.0	232.5	3.76
7	85	119.90	112.00	103.00	42.22	58.0	298.0	4.55
8	82	121.51	113.25	93.75	37.39	64.5	220.5	4.10
9	83	140.04	133.00	120.87	46.79	49.0	279.0	5.10
10	82	158.51	148.00	117.50	58.40	68.0	332.0	6.41
11	62	157.88	147.00	136.75	63.20	59.0	370.0	7.96
12	51	181.98	155.00	126.50	76.96	37.0	395.0	10.67
13	37	241.02	233.00	187.20	109.41	33.0	547.0	17.74
14	18	240.63	202.50	154.75	122.49	72.0	568.5	28.05
15	11	292.54	226.00	193.50	138.67	87.0	513.0	39.86

TABLE 29. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM TOTAL PROTEIN FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	4.62	4.50	4.55	0.46	3.9	6.0	0.04
2	94	5.82	5.80	5.77	0.26	5.4	6.6	0.02
3	90	5.99	6.00	6.07	0.31	5.2	7.2	0.03
4	92	6.25	6.30	6.35	0.28	5.7	7.2	0.02
5	87	6.21	6.20	6.15	0.30	5.4	7.8	0.03
6	92	6.12	6.15	6.05	0.27	5.4	6.9	0.02
7	89	6.13	6.20	6.04	0.21	5.7	6.8	0.02
8	93	6.21	6.20	5.91	0.30	5.4	7.0	0.03
9	88	6.22	6.20	6.13	0.23	5.7	7.1	0.02
10	89	6.19	6.20	6.21	0.27	5.6	7.0	0.02
11	64	6.07	6.10	6.17	0.25	5.4	6.6	0.03
12	49	6.04	6.00	5.97	0.29	5.1	6.6	0.04
13	33	6.13	6.00	5.85	0.43	4.8	7.6	0.07
14	18	5.93	6.00	5.77	0.35	5.1	6.4	0.08
15	12	5.76	5.75	5.57	0.27	5.3	6.4	0.07

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	4.60	4.50	4.40	0.48	3.2	6.3	0.04
2	98	5.81	5.80	5.83	0.27	5.1	6.4	0.02
3	96	5.88	5.90	6.15	0.41	3.2	7.5	0.04
4	94	6.22	6.15	6.16	0.23	5.7	6.7	0.02
5	86	6.18	6.20	6.12	0.24	5.6	6.8	0.02
6	86	6.17	6.15	6.07	0.26	5.7	6.9	0.02
7	87	6.10	6.20	6.10	0.27	4.9	7.0	0.03
8	84	6.22	6.15	5.90	0.28	5.4	7.0	0.03
9	85	6.21	6.20	6.43	0.19	5.7	6.6	0.02
10	82	6.18	6.20	5.97	0.27	5.6	6.8	0.03
11	63	6.15	6.10	6.05	0.23	5.6	6.7	0.02
12	52	6.04	6.10	6.09	0.27	5.2	6.7	0.03
13	36	5.97	6.00	6.05	0.35	5.2	6.9	0.05
14	18	5.86	5.92	6.25	0.49	4.5	6.6	0.11
15	11	5.99	5.90	5.90	0.30	5.6	6.8	0.08

TABLE 30. RESULTS OF STATISTICAL ANALYSIS OF DATA ON SERUM ALBUMIN FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	2.82	2.70	2.62	0.24	2.4	3.6	0.02
2	98	3.29	3.20	3.20	0.18	2.4	3.6	0.02
3	93	3.23	3.30	3.16	0.18	2.6	3.6	0.01
4	94	3.45	3.45	3.47	0.18	2.5	4.2	0.01
5	87	3.41	3.40	3.45	0.19	2.5	4.2	0.02
6	93	3.46	3.45	3.45	0.15	3.0	3.8	0.01
7	91	3.15	3.20	3.09	0.15	2.7	3.6	0.01
8	93	3.26	3.30	3.31	0.18	2.7	3.6	0.01
9	89	3.17	3.20	3.31	0.18	2.7	3.6	0.01
10	89	3.24	3.20	3.16	0.23	2.6	3.6	0.02
11	64	2.99	3.00	2.95	0.24	2.5	3.4	0.03
12	49	2.75	2.80	2.71	0.23	2.3	3.3	0.03
13	35	3.09	3.10	2.96	0.29	2.4	3.9	0.04
14	18	3.11	3.15	2.92	0.31	2.2	3.6	0.07
15	12	2.71	2.70	2.55	0.31	2.3	3.3	0.08

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	2.82	2.70	2.62	0.25	2.0	3.6	0.02
2	99	3.28	3.20	3.16	0.20	2.2	3.6	0.02
3	98	3.19	3.20	3.27	0.23	1.9	3.9	0.02
4	95	3.44	3.45	3.37	0.16	3.0	4.2	0.01
5	87	3.38	3.40	3.37	0.14	2.9	3.7	0.01
6	86	3.46	3.45	3.47	0.14	3.1	3.9	0.01
7	88	3.16	3.20	3.14	0.18	2.5	3.6	0.02
8	87	3.27	3.30	3.30	0.21	2.6	4.2	0.02
9	85	3.17	3.20	3.09	0.18	2.7	3.6	0.02
10	83	3.24	3.20	3.23	0.22	2.8	3.8	0.02
11	64	3.06	3.10	3.17	0.23	2.4	3.6	0.02
12	52	2.78	2.80	2.61	0.23	2.2	3.2	0.03
13	37	3.00	3.00	3.17	0.30	2.4	3.5	0.04
14	18	3.16	3.15	3.15	0.26	2.7	3.6	0.06
15	11	2.83	2.80	2.65	0.21	2.5	3.1	0.06

TABLE 31. RESULTS OF STATISTICAL ANALYSIS DATA ON SERUM GLOBULIN FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	1.79	1.80	1.76	0.33	1.2	3.0	0.03
2	94	2.53	2.60	2.35	0.21	2.2	3.0	0.02
3	90	2.76	2.75	2.89	0.23	2.3	4.2	0.02
4	92	2.79	2.70	2.65	0.22	2.4	3.7	0.02
5	86	2.78	2.80	2.70	0.24	1.8	3.4	0.02
6	92	2.66	2.70	2.45	0.24	1.8	3.3	0.02
7	89	2.97	3.00	3.03	0.19	2.6	3.6	0.02
8	93	2.94	2.85	2.77	0.26	2.4	3.6	0.02
9	88	3.05	3.00	2.98	0.20	2.7	3.5	0.02
10	89	2.95	3.00	2.77	0.26	2.4	3.6	0.02
11	64	3.08	3.10	3.17	0.22	2.4	3.6	0.02
12	49	3.28	3.20	3.15	0.23	2.7	3.8	0.03
13	33	3.05	3.00	3.03	0.34	2.4	4.1	0.05
14	18	2.82	2.77	2.55	0.28	2.4	3.3	0.06
15	12	3.05	3.10	3.27	0.22	2.6	3.5	0.06

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	109	1.77	1.80	1.70	0.32	1.2	3.0	0.03
2	98	2.52	2.60	2.52	0.22	2.0	3.2	0.02
3	96	2.69	2.70	2.59	0.23	1.3	3.6	0.02
4	94	2.77	2.70	2.68	0.18	2.4	3.3	0.01
5	86	2.80	2.80	2.79	0.17	2.4	3.3	0.01
6	86	2.70	2.70	2.62	0.21	2.2	3.4	0.02
7	87	2.93	2.90	2.83	0.18	2.4	3.4	0.02
8	84	2.94	3.00	2.75	0.25	2.1	3.6	0.02
9	85	3.03	3.00	3.02	0.23	2.5	3.7	0.02
10	82	2.93	3.00	2.99	0.27	2.4	3.4	0.03
11	63	3.09	3.10	3.10	0.25	2.4	4.1	0.03
12	52	3.26	3.25	3.20	0.26	2.7	3.9	0.03
13	36	2.96	2.95	2.90	0.25	2.6	3.6	0.04
14	18	2.77	2.70	2.77	0.30	2.2	3.3	0.07
15	11	3.15	3.10	3.02	0.25	2.8	3.7	0.07

TABLE 32. RESULTS OF STATISTICAL ANALYSIS OF ALBUMIN/GLOBULIN RATIOS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	1.62	1.50	1.40	0.30	0.9	2.5	0.03
2	94	1.29	1.30	1.25	0.14	0.8	1.6	0.01
3	90	1.17	1.20	1.20	0.11	0.7	1.4	0.01
4	92	1.25	1.30	1.20	0.12	0.7	1.5	0.01
5	86	1.24	1.20	1.20	0.18	0.7	2.3	0.01
6	92	1.31	1.30	1.43	0.15	1.0	2.0	0.01
7	89	1.06	1.10	1.08	0.10	0.8	1.3	0.01
8	93	1.12	1.10	1.21	0.12	0.8	1.4	0.01
9	88	1.04	1.10	1.08	0.10	0.8	1.3	0.01
10	89	1.10	1.10	1.06	0.15	0.8	1.4	0.01
11	64	0.97	1.00	0.95	0.13	0.7	1.4	0.01
12	49	0.84	0.90	0.90	0.10	0.6	1.0	0.01
13	33	1.02	1.00	0.96	0.16	0.7	1.4	0.02
14	18	1.13	1.10	0.91	0.17	0.8	1.5	0.03
15	12	0.90	0.90	0.82	0.15	0.7	1.2	0.04

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	100	1.63	1.55	1.41	0.27	1.0	2.5	0.02
2	98	1.30	1.30	1.13	0.14	0.7	1.7	0.01
3	96	1.19	1.20	1.16	0.09	0.9	1.5	0.01
4	94	1.26	1.30	1.15	0.11	1.0	1.8	0.01
5	86	1.21	1.20	1.17	0.09	1.0	1.4	0.01
6	86	1.29	1.30	1.28	0.10	1.0	1.5	0.01
7	87	1.08	1.10	1.07	0.09	0.9	1.3	0.01
8	84	1.13	1.10	1.13	0.14	0.7	1.7	0.01
9	85	1.07	1.10	1.08	0.12	0.8	1.3	0.01
10	82	1.11	1.10	1.10	0.15	0.8	1.5	0.01
11	63	0.99	1.00	0.93	0.14	0.6	1.4	0.01
12	52	0.85	0.80	0.75	0.13	0.6	1.2	0.01
13	36	1.01	1.00	1.00	0.14	0.7	1.3	0.02
14	18	1.10	1.15	1.21	0.26	0.3	1.4	0.06
15	11	0.89	0.90	0.87	0.10	0.8	1.1	0.03

Discussion

Inspection of the data confirms that no gross clinical abnormalities due to the experimental treatment are discernible and all parameters fall within accepted physiological normal ranges. However, specific observations concerning the overall pattern of particular parameters are of interest.

Glucose decreased slightly with age for both the exposed and sham-exposed populations. The slight rise in glucose levels apparent during the final sampling may be attributable to heightened arousal due to changes in handling, anesthesia, and sampling technique immediately prior to sample collection. This rise may reflect a transient epinephrine-induced hyperglycemia via its glycogenolytic action.

Blood urea nitrogen appears to have been fairly stable throughout the 25 months of the experiment except during the final two sampling periods, in which the sample sizes involved, coupled with debilitation of a few animals, led to alterations of the mean and error estimates.

Creatinine levels were higher in the immature animals (as measured during the first sampling session, prior to microwave exposure) but were consistent with rapid muscle development. Elevations in creatinine during the final two blood samplings paralleled the BUN increases, presumably owing to the same deteriorating condition of a few animals.

Examination of the electrolytes reveals a stable sodium level throughout the entire lifespan. Potassium appears to have been elevated in the immature animals but to have decreased very gradually with age. The apparent elevation of potassium levels for both treatment groups during the final sampling was due to hemolysis of the collected samples. Chloride appears to have increased as the animals matured, reached a plateau at approximately 4 months of age (session 3), and remained stable throughout the remaining lifespan.

Carbon dioxide levels appear to have been lower in the immature animals during the first session and then to have remained stable throughout the lifetime of the animals.

Uric acid levels were slightly higher in the immature animals but then remained stable throughout their lifetime. The apparent elevation during

the final sampling was due to interference with the colorimetric determination method as a result of hemolysis of the collected samples.

Calcium, as expected, was very tightly maintained throughout the lifespan of the animals.

Phosphorus and alkaline phosphatase levels were highest in the rapidly growing young animals, stabilized with maturity, and remained stable for the remainder of life.

Levels of LDH, SGOT, and SGPT were unremarkable throughout the lifetime of the animals. The apparent rise and fall of SGPT levels is simply a reflection of changes in methodology (as mirrored in the control serum data) and do not reflect any actual change in clinical levels. The apparent rise in LDH and SGOT in the final sessions was the result of sample hemolysis.

Cholesterol and triglyceride levels progressively increased throughout the lifetime of the animals. Although the cholesterol levels remained comparable between the final two samplings, the triglyceride levels for the exposed and sham-exposed animals appeared to differ. Correlation of these results will be made in Volume 9 of this series.

Both the exposed and sham-exposed populations showed a gradual decrease in A/G ratio with increasing age. The overall level of the globulin fractions observed in these barrier-sustained animals is lower than reported from conventional-colony animals.

PROTEIN ELECTROPHORETIC PATTERN AND FRACTIONS

Serum protein electrophoresis (SPE) is not a method for determining specific proteins, but is considered a valuable determination for developing organ panels or health profiles. It is the single most sensitive procedure for detecting monoclonal gammopathies and is used to determine certain proteins such as albumin and immunoglobulins. With a well-resolved system, even small monoclonal or oligoclonal bands can be easily identified, giving evidence of intense immunologic stimulation such as may accompany serious viral infections or tissue necrosis. Individual proteins other than albumin cannot be measured with certainty, because the electrophoretic bands or fractions (albumin, alpha 1 and 2, beta, and gamma) represent a composite of many species of protein. Approximately 15 proteins are present in normal serum in sufficient concentrations to influence the electrophoretic patterns seen. Because several of them have similar mobilities, when stained for protein they give rise to only five to six clear bands or fractions.

From results of electrophoretic fractionations, limited clinical significance can be associated with small variations from the normal range of values. Discrimination increases with serial patterns obtained at 6- or 12-week intervals, as in this study. These were reviewed for detection of possible progressive changes or relative consistency and were thus more meaningful than a single examination. Changes in the electrophoretic patterns are a useful component of the health profile in defining the pathophysiological mechanisms of certain abnormalities. In a few diseases, changes in SPE are very characteristic. For example, in the nephrotic syndrome, albumin and other lower-molecular-mass proteins are excreted in the urine. Some immunoglobulin-G is also lost; and this loss, coupled with a rise in concentration of larger molecules such as the alpha-2 macroglobulin and heptoglobins, leads to faint albumin and gamma bands associated with an intense alpha-2 band.

In the rat serum, high concentration of one specific kind of immunoglobulin suggests the existence of either a benign or malignant proliferation of plasma cells (multiple myeloma). These cells are genetically identical and secrete an identical kind of immunoglobulin. These monoclonal immunoglobulin disorders are usually characterized by an

intense extra band anywhere in the gamma and beta regions or even in the alpha-2 region of the SPE pattern. Primary or secondary immune-deficiency may be characterized by a deficiency of immunoglobulin and is suggested by a faint or absent gamma band.

The change in serum protein composition that follows tissue damage (as occurs in injury), acute glomerulonephritis, myocardial infarction, or acute infection is referred to as the acute phase reaction. The most marked change is in the increase in alpha-2 globulin, which is mainly due to a two- or threefold increase in the level of heptoglobin. Other less frequent changes are a fall in albumin and a rise in alpha 1, due to increases in alpha-1 antitrypsin and orosomucoid. Gamma globulins may increase at a later stage of the disease.

Increased concentration of serum immunoglobulins, due to antibody synthesis, causes a general increase in the intensity of the gamma band, as is observed in chronic disease (portal cirrhosis). The concentration of immunoglobulin A is most frequently increased. Since IgA is predominantly of beta mobility, a characteristic beta-gamma bridging or fusion results.

Combinations of these responses may occur; for example, a monoclonal immunoglobulin disorder may be combined with an immune deficiency, or a reaction to tissue destruction may be combined with increased synthesis of polyclonal immunoglobulins.

Methods

A microzone electrophoresis system (produced by Beckman Instruments, Incorporated, Fullerton, CA) was used in the protein electrophoresis. Eight serum samples were applied to a single cellulose acetate membrane (5.7 x 12.7 mm) with the applicator. The membrane was mounted in a single electrophoresis chamber containing 700 ml of buffer (Beckman B-2 barbital buffer with pH of 8.6). The samples were then electrophoresed for 25 min at 250 V, after which the membranes were stained with Ponceau-S staining solution and clarified. The results were well-defined serum-protein fractions on a transparent background. Quantitation, accomplished with a computing densitometer, provided a scan of the optical density at 525 nm and computed the area under the portions of the scan corresponding to the various protein fractions.

Results

The results of the serum-protein electrophoresis are presented in Figs. 35 through 38 and are based on the serum chemistry determination of total protein. A summary of the results of the statistical evaluation of the data is presented for each parameter in Tables 33 through 36. Inspection of the correlation matrices indicates that run-to-run correlation for any parameter was uniformly low. None of the pairwise t-test comparisons of the means were significant at the .05 level; therefore, multivariate analysis of the data was not performed.

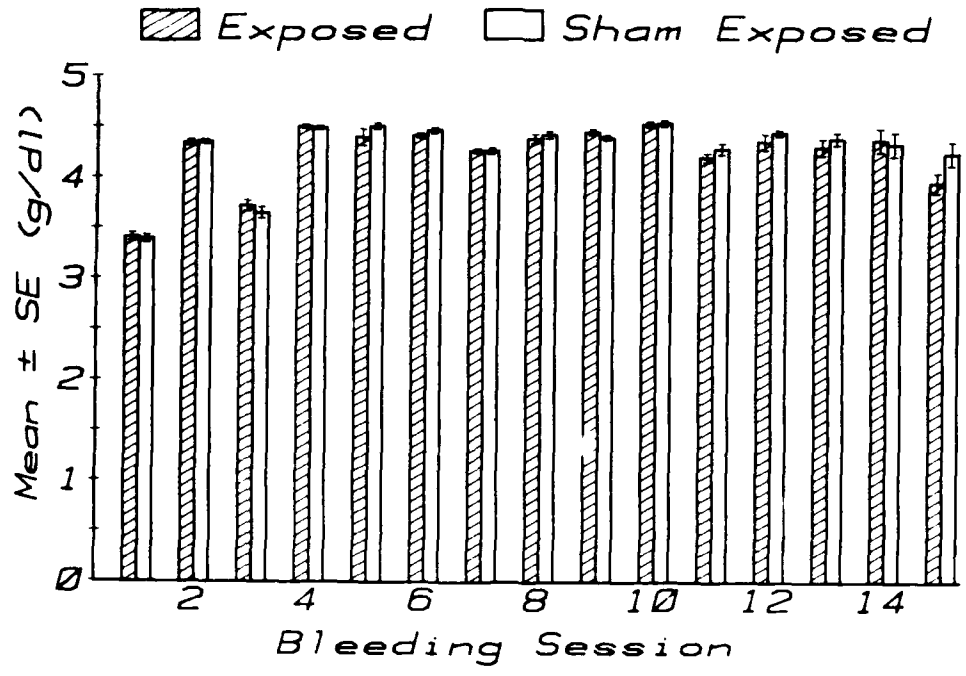

Fig. 35. ALBUMIN fractions for exposed and sham-exposed animals for 15 sampling sessions.

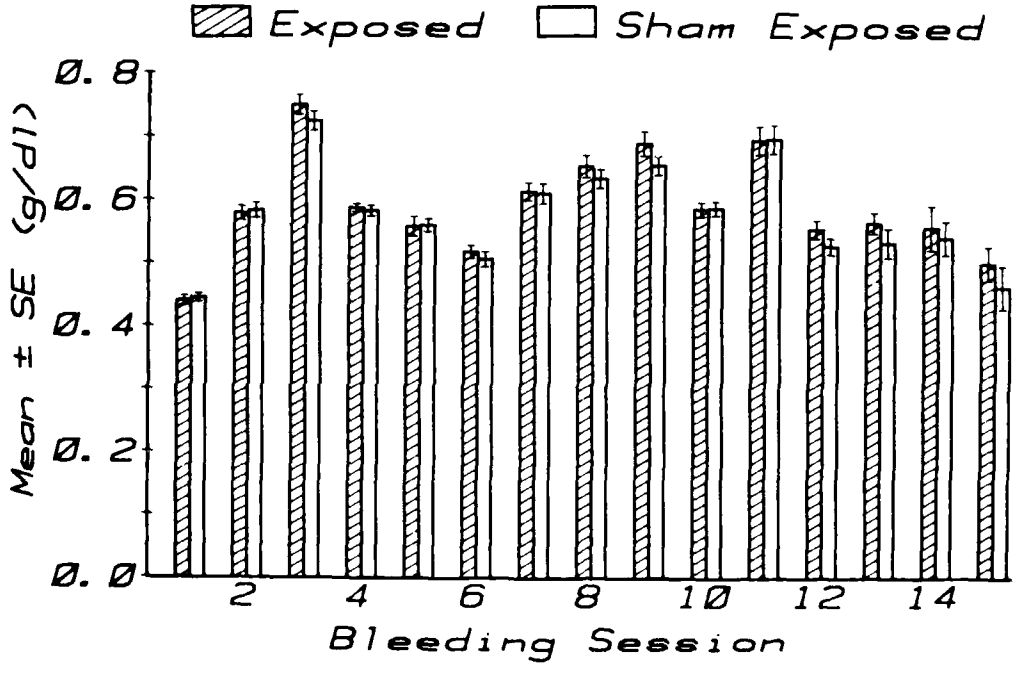

Fig. 36. ALPHA-1 and -2 PROTEIN fractions for exposed and sham-exposed animals for 15 sampling sessions.

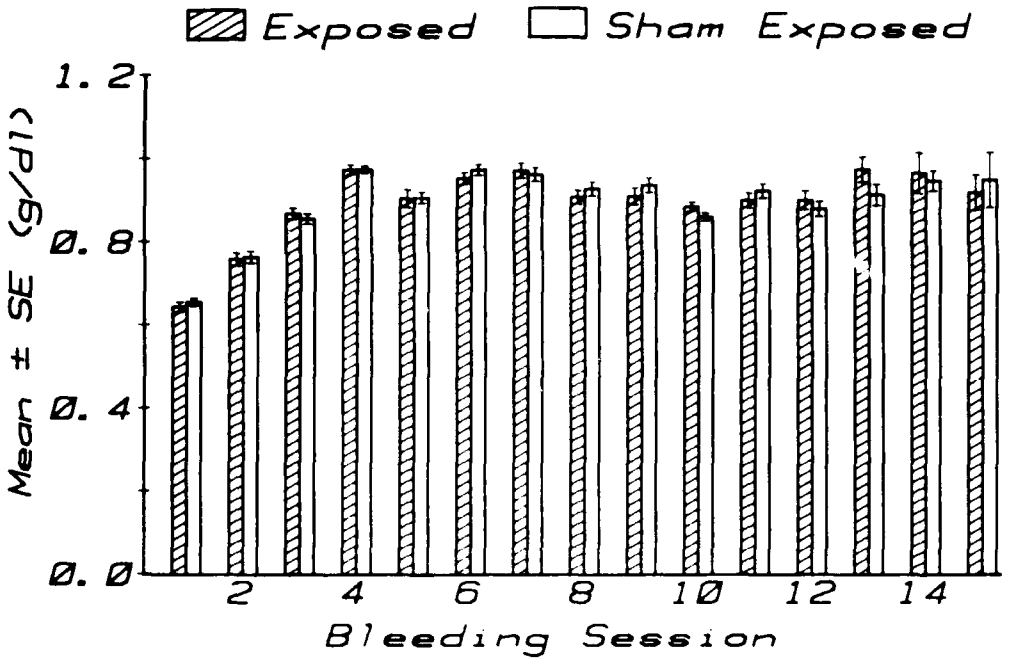

Fig. 37. BETA PROTEIN fractions for exposed and sham-exposed animals for 15 sampling sessions.

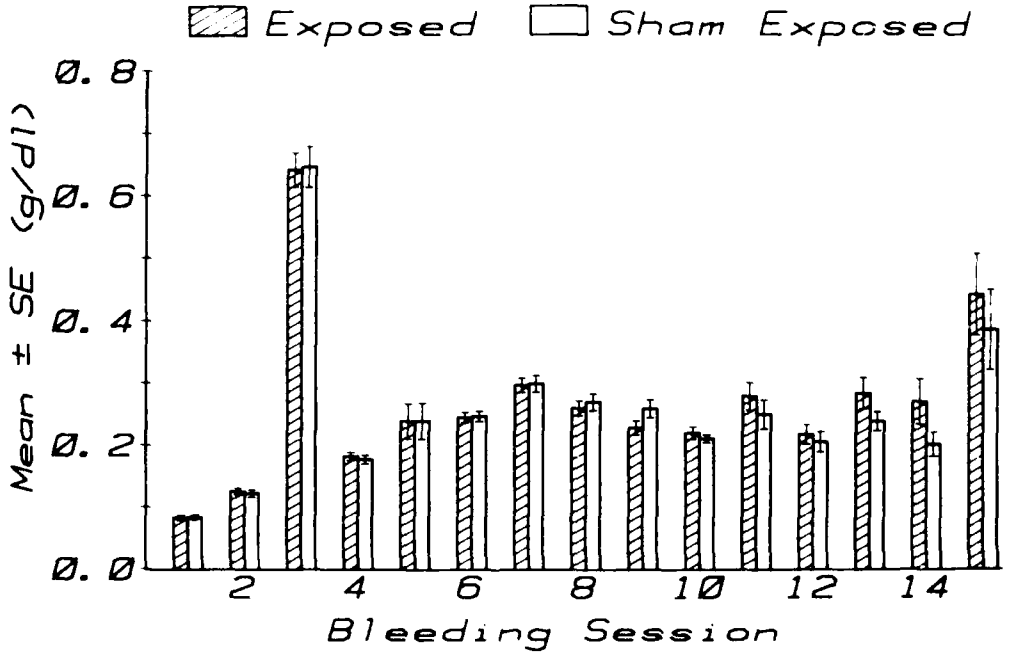

Fig. 38. GAMMA PROTEIN fractions for exposed and sham-exposed animals for 15 sampling sessions.

TABLE 33. RESULTS OF STATISTICAL ANALYSIS OF ALBUMIN FRACTIONS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	93	3.41	3.33	3.33	0.42	1.1	4.6	0.04
2	88	4.35	4.35	4.56	0.29	3.1	5.0	0.03
3	90	3.72	3.66	3.84	0.53	2.5	5.0	0.06
4	91	4.51	4.49	4.60	0.22	4.1	5.2	0.02
5	85	4.40	4.47	4.34	0.76	0.0	5.7	0.08
6	87	4.42	4.43	4.54	0.24	3.8	5.0	0.03
7	89	4.26	4.25	4.28	0.23	3.4	5.0	0.02
8	91	4.38	4.43	4.55	0.45	2.2	5.6	0.05
9	87	4.44	4.40	4.54	0.30	3.8	5.3	0.03
10	87	4.52	4.53	4.65	0.30	3.7	5.4	0.03
11	63	4.19	4.22	4.22	0.37	3.3	4.9	0.05
12	43	4.35	4.39	4.66	0.56	1.6	5.7	0.08
13	29	4.30	4.33	5.02	0.45	2.8	5.0	0.08
14	17	4.37	4.33	4.14	0.50	3.5	5.8	0.12
15	11	3.95	3.96	4.39	0.34	3.5	4.4	0.10

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	3.39	3.33	3.33	0.39	2.4	4.7	0.04
2	93	4.36	4.35	4.50	0.22	3.8	5.0	0.02
3	94	3.66	3.60	3.72	0.57	1.9	5.1	0.06
4	91	4.49	4.47	4.43	0.21	3.9	5.0	0.02
5	85	4.51	4.48	4.40	0.31	3.9	5.3	0.03
6	80	4.47	4.43	4.38	0.28	3.7	5.5	0.03
7	85	4.26	4.28	4.48	0.29	3.3	5.3	0.03
8	82	4.42	4.43	4.26	0.34	3.3	5.5	0.04
9	84	4.39	4.37	4.29	0.25	3.7	5.2	0.03
10	79	4.53	4.48	4.46	0.28	4.1	5.5	0.03
11	59	4.28	4.38	4.56	0.44	2.9	5.2	0.06
12	49	4.44	4.47	4.59	0.23	3.5	4.8	0.03
13	30	4.38	4.39	4.38	0.34	3.2	5.3	0.06
14	17	4.33	4.45	4.56	0.50	2.6	4.7	0.12
15	10	4.23	4.29	4.83	0.39	3.5	4.8	0.12

TABLE 34. RESULTS OF STATISTICAL ANALYSIS OF ALPHA-1 AND -2 PROTEIN FRACTIONS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	93	0.44	0.45	0.45	0.08	0.3	0.7	0.01
2	88	0.58	0.56	0.70	0.11	0.3	0.8	0.01
3	90	0.75	0.74	0.73	0.15	0.4	1.3	0.02
4	91	0.59	0.58	0.57	0.07	0.5	0.8	0.01
5	85	0.56	0.57	0.73	0.15	0.0	0.8	0.02
6	87	0.52	0.50	0.50	0.10	0.4	1.0	0.01
7	89	0.61	0.61	0.54	0.14	0.2	1.2	0.01
8	91	0.65	0.62	0.55	0.17	0.2	1.4	0.02
9	87	0.69	0.68	0.71	0.18	0.3	1.7	0.02
10	87	0.59	0.58	0.62	0.11	0.1	0.9	0.01
11	63	0.69	0.69	0.69	0.18	0.2	1.2	0.02
12	48	0.55	0.55	0.63	0.10	0.3	0.8	0.01
13	29	0.57	0.58	0.59	0.09	0.4	0.8	0.02
14	13	0.56	0.51	0.62	0.13	0.4	0.9	0.04
15	11	0.50	0.46	0.46	0.09	0.3	0.6	0.03

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	0.45	0.45	0.45	0.07	0.2	0.6	0.01
2	93	0.58	0.59	0.62	0.12	0.3	0.8	0.01
3	94	0.72	0.73	0.73	0.15	0.1	1.1	0.02
4	91	0.58	0.58	0.62	0.09	0.3	0.9	0.01
5	85	0.56	0.58	0.58	0.10	0.2	0.9	0.01
6	81	0.51	0.49	0.55	0.11	0.3	1.2	0.01
7	85	0.61	0.62	0.63	0.15	0.2	1.3	0.02
8	82	0.63	0.62	0.62	0.14	0.3	1.4	0.02
9	84	0.65	0.67	0.58	0.14	0.3	1.0	0.02
10	79	0.59	0.58	0.58	0.10	0.1	0.8	0.01
11	59	0.70	0.70	0.79	0.18	0.2	1.2	0.02
12	49	0.53	0.55	0.56	0.09	0.3	0.7	0.01
13	30	0.53	0.54	0.56	0.13	0.3	1.0	0.02
14	14	0.54	0.52	0.64	0.10	0.4	0.8	0.03
15	10	0.46	0.47	0.41	0.12	0.2	0.7	0.03

TABLE 35. RESULTS OF STATISTICAL ANALYSIS OF BETA PROTEIN FRACTIONS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	93	0.64	0.63	0.63	0.11	0.2	0.9	0.01
2	88	0.76	0.75	0.70	0.15	0.4	1.3	0.02
3	90	0.87	0.88	0.96	0.13	0.4	1.2	0.01
4	91	0.97	0.96	1.01	0.11	0.5	1.3	0.01
5	85	0.90	0.92	0.92	0.20	0.0	1.5	0.02
6	87	0.95	0.94	1.01	0.13	0.5	1.4	0.01
7	89	0.97	0.99	1.02	0.18	0.3	1.4	0.02
8	91	0.91	0.92	0.90	0.16	0.2	1.3	0.02
9	87	0.91	0.92	0.88	0.19	0.2	1.4	0.02
10	87	0.88	0.87	0.87	0.10	0.6	1.1	0.01
11	63	0.90	0.92	0.93	0.15	0.5	1.3	0.02
12	48	0.90	0.88	0.88	0.16	0.6	1.3	0.02
13	29	0.97	0.94	0.94	0.17	0.6	1.4	0.03
14	18	0.96	0.95	1.46	0.22	0.5	1.5	0.05
15	11	0.91	0.86	0.81	0.15	0.8	1.2	0.04

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	0.66	0.64	0.72	0.10	0.3	0.9	0.01
2	93	0.76	0.76	0.81	0.14	0.4	1.3	0.01
3	94	0.86	0.87	0.94	0.12	0.4	1.1	0.01
4	91	0.97	0.97	1.01	0.09	0.8	1.3	0.01
5	85	0.91	0.92	0.94	0.13	0.3	1.2	0.01
6	81	0.97	0.94	0.90	0.12	0.7	1.5	0.01
7	85	0.96	0.96	0.93	0.15	0.2	1.3	0.02
8	82	0.93	0.93	0.92	0.16	0.3	1.2	0.02
9	84	0.93	0.94	1.02	0.17	0.2	1.5	0.02
10	79	0.86	0.86	0.90	0.09	0.6	1.2	0.01
11	59	0.92	0.92	0.96	0.14	0.6	1.3	0.02
12	49	0.88	0.88	0.88	0.13	0.6	1.3	0.02
13	30	0.91	0.92	0.92	0.14	0.6	1.1	0.03
14	18	0.94	0.92	0.86	0.11	0.8	1.2	0.02
15	10	0.94	0.86	1.36	0.22	0.7	1.4	0.07

TABLE 36. RESULTS OF STATISTICAL ANALYSIS OF GAMMA PROTEIN FRACTIONS FOR EXPOSED AND SHAM-EXPOSED RATS FOR 15 SAMPLING SESSIONS

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	93	0.08	0.09	0.05	0.04	0.0	0.2	0.00
2	88	0.13	0.12	0.12	0.05	0.0	0.3	0.01
3	90	0.64	0.63	0.32	0.26	0.1	1.3	0.03
4	91	0.18	0.18	0.18	0.07	0.0	0.4	0.01
5	83	0.24	0.23	0.31	0.26	0.0	2.3	0.03
6	87	0.24	0.24	0.25	0.08	0.1	0.5	0.01
7	89	0.30	0.29	0.24	0.11	0.1	0.8	0.01
8	91	0.26	0.25	0.26	0.12	0.1	0.7	0.01
9	87	0.23	0.24	0.25	0.11	0.0	0.5	0.01
10	87	0.22	0.19	0.19	0.10	0.0	0.5	0.01
11	63	0.28	0.24	0.12	0.18	0.1	0.9	0.02
12	48	0.22	0.19	0.18	0.11	0.0	0.5	0.02
13	29	0.28	0.24	0.18	0.14	0.1	0.6	0.03
14	18	0.27	0.22	0.17	0.16	0.1	0.7	0.04
15	11	0.44	0.50	0.50	0.23	0.2	1.0	0.07

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	98	0.08	0.09	0.09	0.04	0.0	0.2	0.00
2	93	0.12	0.12	0.12	0.06	0.0	0.3	0.01
3	94	0.65	0.62	0.92	0.32	0.0	1.3	0.03
4	90	0.18	0.18	0.18	0.07	0.0	0.3	0.01
5	85	0.24	0.22	0.26	0.27	0.0	2.4	0.03
6	81	0.25	0.24	0.18	0.08	0.1	0.5	0.01
7	85	0.30	0.29	0.32	0.13	0.1	0.7	0.01
8	82	0.27	0.25	0.25	0.13	0.0	0.8	0.01
9	84	0.26	0.24	0.24	0.14	0.0	1.0	0.01
10	79	0.21	0.19	0.19	0.06	0.1	0.3	0.01
11	59	0.25	0.20	0.12	0.18	0.0	1.0	0.02
12	49	0.21	0.19	0.19	0.12	0.1	0.9	0.02
13	29	0.24	0.24	0.18	0.08	0.1	0.4	0.02
14	18	0.20	0.18	0.18	0.09	0.1	0.4	0.02
15	10	0.39	0.32	0.24	0.22	0.2	0.9	0.06

Discussion

Electrophoretic patterns and absolute protein fraction values show no significant changes between the exposed and sham-exposed groups. This indicates that the level of RFR exposure had no apparent effect on various organ-system functions that contribute to serum protein concentrations. Production of neither albumin by the liver nor globulin fractions by the lymphoreticular system was altered. Normal levels of alpha fractions reflect none of the increases usually seen in tissue destruction nor the decreases often associated with renal glomerular damage.

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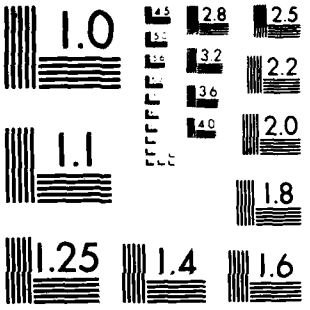

THYROXINE

Elevated contents of serum cholestrol and triglycerides and a mild normochromic, normocytic anemia are nonspecific traits suggestive of hypothyroidism and do not occur in all hypothyroid animals. The physiological and quantitative differences in thyroid functions between man and rats have not been fully investigated. The influence of alterations in thyroid-binding proteins has not been as thoroughly investigated in rats as in man. Until all variables related to thyroid-function tests in rats have been thoroughly evaluated, the reliability of such tests for adequate prediction of the status of the rat thyroid gland will be questioned.

The thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), are active in controlling the growth, development, and proper function of organ systems and in regulating and maintaining normal metabolism throughout the body. The synthesis of T_4 and T_3 in the thyroid gland and their release into the circulatory system are regulated by a complex feedback mechanism involving the hypothalamus and the anterior pituitary hypophysis. Most of the circulating T_4 and T_3 are bound to T_4-binding globulin; some T_4 is bound to T_4-binding prealbumin, and to a lesser degree both hormones are bound to albumin.

Much smaller amounts of these hormones circulate in a free form; i.e., they are not bound to proteins. The free forms of T_4 and T_3 are believed to be the functional or active forms of these hormones. The levels of the T_4-binding proteins, the amount of T_4 that is bound to these proteins, and the amount of free T_4 usually have a fairly constant relationship. If we assume that the levels of T_4-binding proteins are within normal limits, an accurate measure of the level of serum T_4 is accepted as a valuable tool for assessing the function of the thyroid gland and the organs involved in regulating the thyroid function. Thyroxine has been a parameter examined in rats exposed to microwave radiation (Lotz, 1979, and Michaelson et al., 1977).

Methods

The radioimmunoassay for T_4 is extremely sensitive, and small quantities of serum (25 µg) are required for the test. In the test procedure, the rat serum is mixed with radioactively labeled T_4 (^{125}I) and 8-anilino-1-naphthalene sulfonic acid (ANS); then an immobilized T_4 antiserum is added. The mixture is allowed to incubate at room temperature. The ANS displaces the T_4 from the serum proteins. During incubation, the displaced T_4 competes with the labeled T_4 for the immobilized T_4 antibodies on the basis of their relative concentrations.

The quantity of labeled T_4 that binds with the antibody is inversely related to the amount of unlabeled endogenous T_4 present in the serum. After incubation the mixture is centrifuged, and the immobilized T_4-antibody complex is concentrated at the bottom of the tube in the form of a pellet. The unbound T_4 in the supernatant is decanted, and the radioactivity associated with the pellet is counted. A standard curve is prepared from precalibrated T_4 standards. From this curve, the concentration of T_4 in the rat serum is determined.

The procedure used is a modified quantimmune-T_4-RIA method that is based on the principles of radioimmunoassay described by Berson and Yalow (1960). A Packard autogamma scintillation spectrometer 5130 is used. The procedural steps comprise:

1. Labeling 12- x 75-mm reaction tubes for each standard, including the zero, each control, and each sample.
2. Adding 25 µg of each standard, control, or experimental sample to the appropriate tubes.
3. Adding 500 µg of tracer/dissociating-agent solution to all tubes (standards, control, and samples).
4. Preparing a total-counts tube by adding 300 µg of tracer/dissociating-agent solution to a 12- x 75-mm reaction tube, to be set aside until Step 10.
5. Briefly mixing the vial of T_4 immunobeads, adding 500 µg of the T_4 immunobeads to each tube, and mixing each tube.
6. Incubating all tubes at 37^{o} for 40 min.

7. Centrifuging of all tubes for 15 min at 3000 rpm to pack the T_4 immunobeads on the bottom of the tube; then proceeding IMMEDIATELY to the next step.

8. Discarding the supernatants by smoothly inverting the tubes into a convenient container as they are removed from the centrifuge. Removing the last drop by GENTLY blotting the tube rim on a paper towel.

9. Centrifuging for 15 minutes.

10. Counting each tube, including the total-counts tube, for 1 min, and recording the counts.

The samples are run with a zero, 1.0, 2.5, 5.0, and 10.0 standards and three commercial controls at the beginning and end of the run (low 1.4 $\pm$ .2, medium 7.8 $\pm$ .5, and high 13.6 $\pm$ .7), and three pooled (beginning, middle, and end of run) rat sera. Standard curves are plotted and values determined by a computer program with a log-log plot.

Results

Figure 39 presents comparisons of the T_4 contents for exposed and sham-exposed groups. Analysis with Student's *t*-test of the mean differences between the groups indicated that none of the pairwise comparisons were significant at the .05 level. Data did reveal that both groups showed the expected decrease in T_4 with age. Table 37 is a summary of the results of statistical analysis of the data for all sampling sessions in which T_4 was analyzed.

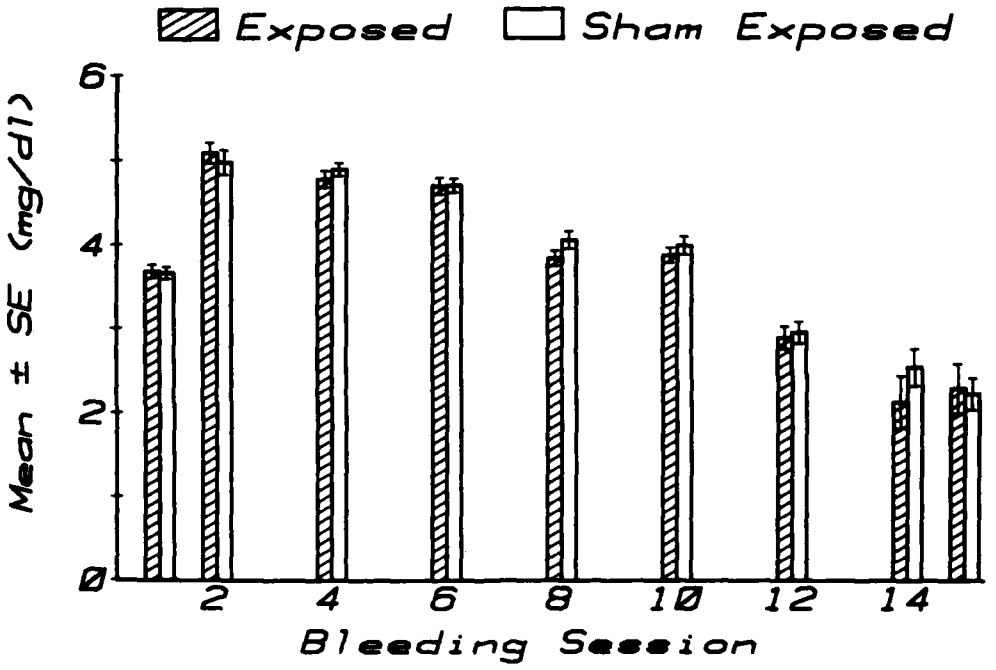

Fig. 39. Comparison of THYROXINE data for exposed and sham-exposed animals for sessions for which analysis was made.

TABLE 37. RESULTS OF STATISTICAL ANALYSIS OF THYROXINE DATA FOR EXPOSED AND SHAM-EXPOSED RATS FOR SAMPLING SESSIONS FOR WHICH ANALYSIS WAS MADE

EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	90	3.68	3.65	3.49	0.78	1.7	5.8	0.08
2	96	5.09	5.30	5.36	1.22	0.0	6.6	0.12
4	91	4.77	4.80	4.53	0.96	0.0	6.6	0.10
6	71	4.69	4.67	4.64	0.85	2.4	6.8	0.10
8	85	3.83	3.87	3.82	0.85	1.3	5.7	0.09
10	89	3.87	3.90	4.01	0.86	1.6	5.9	0.09
12	48	2.89	2.80	2.22	0.95	1.2	4.6	0.13
14	16	2.11	1.95	3.41	1.29	0.0	4.1	0.31
15	12	2.28	1.85	2.00	1.07	1.3	4.1	0.29

SHAM EXPOSED

Run	N	Mean	Med	Mode	SD	Min	Max	SE
1	97	3.65	3.60	3.58	0.73	2.1	5.5	0.07
2	95	4.97	5.30	5.60	1.45	0.0	6.9	0.14
4	94	4.88	4.90	5.02	0.76	3.2	6.4	0.07
6	63	4.70	4.73	4.72	0.67	2.4	6.3	0.08
8	80	4.05	4.10	4.41	0.94	0.8	6.3	0.10
10	80	3.98	3.95	3.72	0.98	1.9	7.0	0.10
12	48	2.95	2.85	2.46	0.92	1.2	5.4	0.13
14	16	2.53	2.60	2.30	0.92	0.8	3.8	0.22
15	11	2.21	2.10	1.75	0.66	1.3	3.1	0.19

Discussion

The T_4 levels of the exposed and sham-exposed animals did not differ significantly. The absolute level developed to a maximum in young animals and decreased gradually with increasing age. This age-related decrease in T_4 appears to reflect general metabolic activity because it developed inversely with the age-related increase of cholesterol and triglyceride. This sensitive balance reflects the absence of effect not only on the thyroid gland, but also on the entire hypothalamic-pituitary-thyroid feedback mechanism and on the tissue metabolism which requires a normal response to hormones of thyroid origin.

This result is significant because there is no indication of any stress phenomenon that would inhibit thyroid secretion. The mechanisms responsible for stress inhibition of thyroid secretion are complex. Secretion of the thyroid-stimulating hormone is apparently inhibited by stress effects mediated by the hypothalamus or pituitary and is independent of the adrenal glands. Also, stress-induced vasoconstriction in the thyroid gland apparently decreases thyroid hormone release (Ganong, 1963). The absence of any detectable alteration in this intricate cycle indicates that the RFR exposure had no adverse effect on any phase of the cycle.

SUMMARY AND CONCLUSIONS

Purported adverse effects on health after long-term exposure to pulsed microwave radiation were investigated in this study. In an attempt to detect and document any cumulative effects on general health and longevity of the exposed animal population, several biochemical and hematological parameters were monitored: serum chemistry components, hematological constituents, protein electrophoretic patterns and fractions, and thyroxine levels. Two hundred Sprague-Dawley rats, divided equally into exposed and sham-exposed groups, were sampled for blood every 6 weeks for the first year and then every 12 weeks until the project was terminated after 25 months of exposure.

Eleven hematological parameters, indices, and absolute cell counts for the leukocytes were analyzed statistically. Multivariate analyses with the Hotelling T^2 statistic on a truncated data set indicated no overall difference between the exposed and sham-exposed populations. Individual t-test comparisons for all parameters for each of the 15 sampling sessions indicated a significant reduction in the absolute eosinophil count for the exposed population during session 2 and marginally significant reductions in absolute neutrophil count during sessions 2 and 3. None of the other individual comparisons were significant. These findings indicate that, despite the 25-month duration of exposure, no detectable effects were produced in the bone marrow erythropoietic cells or in the juxtaglomerular apparatus of the kidney and its production of erythropoietin.

Measurements for 21 serum chemical constituents were performed on serum samples collected from all 15 sampling sessions. The serum chemistry tests were sensitive enough to detect population changes due to aging. Statistical analysis of the data by the Student's t-test did not indicate any differences between the exposed and sham-exposed groups.

Electrophoresis of the serum proteins revealed no significant changes in the electrophoretic patterns and absolute protein fractions between the population groups. Both populations showed a gradual decrease in the albumin/globulin ratio with increasing age, and the overall level of globulin fractions observed in the barrier-sustained animals was lower than reported from conventional-colony animals. The RFR exposure had no

apparent effect on the functioning of various organ systems contributing to serum protein concentrations.

Thyroxine levels did not differ significantly between the exposed and sham-exposed animals. Thus, there was no effect from RFR exposure on the entire hypothalamic-pituitary-thyroid feedback mechanism. The absolute level of serum thyroxine developed to a maximum in young animals and decreased gradually as they aged. The correlation of this age-related decrease in thyroxine levels with increasing cholesterol and triglyceride levels indicates it to be a reliable indicator of metabolic activity in the rat.

The major conclusion that can be reached from the results of evaluations of the hematology, serum chemistry, protein electrophoretic patterns and fractions, and thyroxine levels is that any significant alterations of these parameters during the lifetime of the exposed animals were to be expected with age and were not due to exposure to pulsed microwave radiation.

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